

The Pharmacology of d-Lysergic Acid Diethylamide (LSD)

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by

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“It is my great concern to separate psychedelics from the ongoing debates about drugs, and to highlight the potential inherent to these substances for self-awareness, as an adjunct in therapy, and for fundamental research into the human mind.”

Albert Hofmann

“LSD is a catalyst or amplifier of mental processes. If properly used, it could become something like the microscope or telescope of psychiatry. Whether or not LSD research and therapy will return to society, the discoveries that psychedelics made possible have revolutionary implications for our understanding of the psyche, human nature, and the nature of reality.”

Stanislav Grof

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1. Overview

My PhD thesis consisted of two different scientific parts, each supervised by one of my advisors, Prof. Dr. med. Matthias Liechti and Prof. Dr. sc. nat. Katharina Rentsch.

One part was to develop *liquid chromatography tandem mass spectrometry* (LC-MS/MS) methods to measure *d-lysergic acid diethylamide* (LSD) and its main metabolites in plasma, serum, and urine samples. We established the pharmacokinetics of LSD and collected data from emergency toxicological cases. Therefore we have developed and validated two analytical methods using LC-MS/MS which resulted in several publications. All analytical work was performed in the Toxicology Lab of the Laboratory Medicine at the University Hospital Basel under the supervision of Prof. Dr. sc. nat. Katharina Rentsch.

The second part included planning, conduction, and analysis of clinical phase I trials with LSD. We investigated the acute psychological and physiological effects of LSD in healthy humans what resulted in several publication. These projects were supervised by Prof. Dr. med. Matthias Liechti at the Department of Clinical Pharmacology and Toxicology of the University Hospital Basel. One LSD study included a *functional magnetic resonance imaging* (fMRI) assessment, to investigate the neural correlates of altered states of consciousness and emotion processing under the influence of LSD. The fMRI study was done in close collaboration with the team of Prof. Dr. med. Stefan Borgwardt from the Department of Psychiatry of the University of Basel.

2. Introduction

2.1 Background

Lysergic acid diethylamide (LSD) is a semisynthetic compound related to its precursors lysergic acid and lysergic acid amide which are naturally occurring in different fungi (e.g. *claviceps purpurea*) and plant seeds (e.g. *argyrea nervosa*). The chemical structure of LSD is related to the endogenous neurotransmitter serotonin and other psychedelic drugs such as psilocin, the active compound of the “magic mushrooms” (e.g. *psilocybe cubensis*), and dimethyltryptamine, the active compound of “ayahuasca” (Figure 1). The synthesis of LSD yields four stereoisomeric alkaloids, d- and l-LSD, and d-iso-LSD and l-iso-LSD, whereof only d-LSD possesses the powerful mind-altering effects in animals and humans (1-3). d-LSD is one of the most potent substances, doses above 0.01 mg (10 µg) already produce measurable effects, and from 40 µg upwards induce intense behavioral and perceptual alterations (4, 5). LSD interacts with several brain receptors. Specifically, LSD binds to several subtypes of the serotonin receptor (5-HT_{2A}, 5-HT_{1A}, 5-HT_{2C}), has additional affinity for dopamine D₁ and D₂ receptors (6-8), and indirectly alters glutamatergic neurotransmission via the 5-HT_{2A} receptor (9). The 5-HT_{2A} receptor is also considered the receptor that primarily mediates the hallucinogenic effects of LSD and other serotonergic hallucinogens including psilocin and dimethyltryptamine (10-13). Research with a hallucinogen like LSD always raises some safety concerns. However, LSD possesses little if any abuse liability, is not self-administered by animals, and there is no human LSD dependence syndrome (14). Repeated LSD administration leads to pronounced tolerance to its psychological and physiological effects in less than seven days (15, 16). Further, there is cross-tolerance after repeated administration of psilocybin and other LSD derivatives in humans (17, 18). The tolerance is transient and absent three days after discontinuation. Long-term use in humans is not associated with any evidence of generalized brain damage related to the number of LSD consumptions (19). The chance of precipitating a long-term psychotic reaction is limited to subjects with a personal or familiar history of psychotic disorders (20). Under controlled and supportive conditions, the LSD experience may even have lasting positive effects on attitude and personality (21).

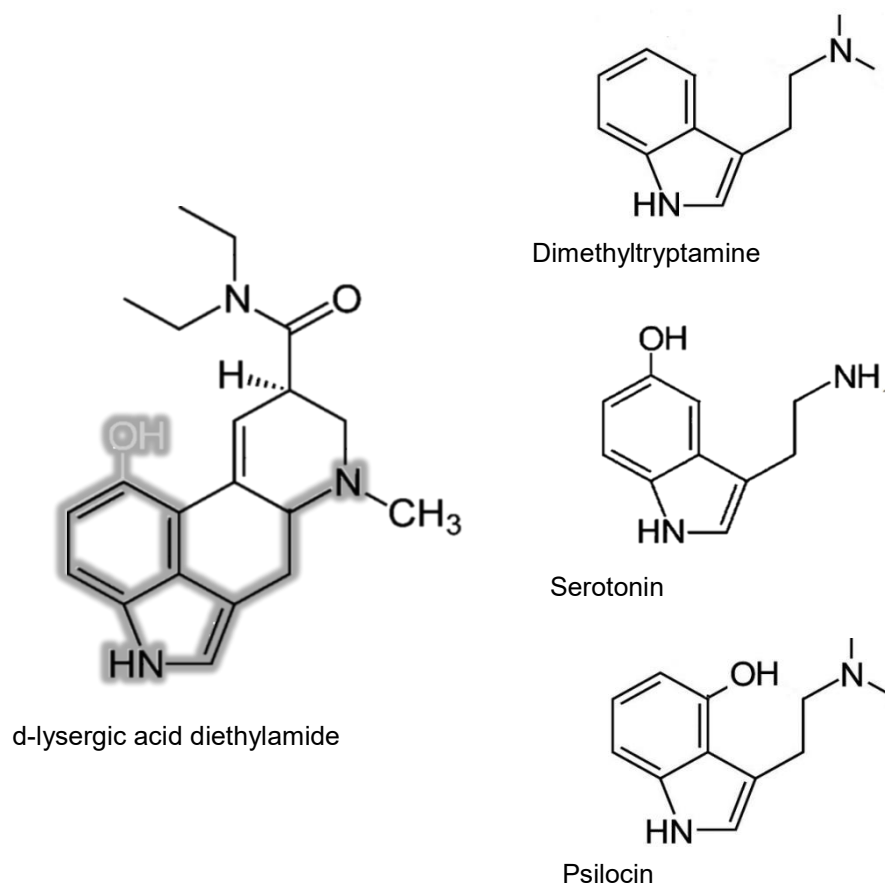


Figure 1 gives the structure of d-lysergic acid diethylamide, the neurotransmitter serotonin (embedded), and the hallucinogens dimethyltryptamine and psilocin.

2.2 The History of LSD

LSD was first synthesized in 1938 here in Basel, and its highly specific actions on the brain and human consciousness were discovered by chance by Albert Hofmann. On the 16th of April in 1943, he decided to resynthesize LSD to repeat tests at the pharmacological department of Sandoz. He got contaminated by accident, and suddenly felt a strong restlessness combined with a slight dizziness whereon he interrupted his work and returned home. He described the following hours as a “not-unpleasant intoxicated dreamlike state, with very stimulated imagination and kaleidoscope-like play of colors” (22). On the 19th of April 1943 he decided to do a self-experiment with 250 µg of the d-LSD tartrate salt. He described the following trip as follows: *“A demon had invaded me and had taken possession of my body, mind and soul. I jumped up and screamed in order to free myself from him, but then sank down again powerless on the sofa. A dreadful fear grasped me that I was becoming insane. I was taken to another world, another place, another time. My body seemed to me to be without sensation, lifeless, strange. Was I dying? Was this the transition? Then, the horror softened and gave way to a feeling of fortune and gratitude, the more normal perceptions and thoughts returned and my assurance increased that the danger of insanity was conclusively past. Now I gradually began to enjoy the unprecedented colors and plays of shapes that persisted behind my closed eyes. It was particularly remarkable how every acoustic perception...became transformed into optical perceptions. Exhausted I then slept and woke up the next morning with a clear head, even though still somewhat tired physically. A sensation of well-being and renewed life flowed through me.”*(22) He wrote a report about his experience to his seniors who repeated his self-experiment, although with lower doses.

From 1949-1966, d-LSD tartrate (LSD-25) was marketed by Sandoz under the brand name Delysid®, and was mostly used in basic psychiatric research and psychotherapy (9, 23-26). Its subjective psychotomimetic effects were compared to those in patients with schizophrenia and led to its use as an experimental substance for model psychosis (2, 27-29). It was thus provided to psychiatrists and researchers with the purpose to study these psychotic phenomena and giving them the possibility of gaining insight into the subjective character of mental disorders.

Soon, a potential therapeutic use was recognized and led to first therapeutic studies at the Psychiatric University Hospital in Zurich (30). In the following years, beneficial effects were documented in the treatment of alcoholism (31), anxiety associated with terminal illness (26, 32, 33) and in the treatment of cluster headache (34).

In hand with the use in a therapeutic setting, LSD was also investigated in social/group settings. Social cognition including emotion recognition and empathy describes the ability to infer another's thoughts, feelings, and intentions and is thus a highly relevant topic not only for social interactions but especially for its use in a psychotherapeutic setting. However, various studies examining social interactions under the influence of LSD showed inconsistent results. This was not surprising, since experiments were carried out in small groups (3 or 4 subjects), in different populations (healthy, alcoholics, addicts, reformatory inmates or schizophrenics) and with variable doses (25 – 200 µg) (35-39). Further, social effects were measured using a variety of tools and included investigations of social perception (rating of liking others in the group or being liked by them) (39), prosocial effects like increased solidarity, tension release, and decreased antagonism (38). Subject's social interactions within the group settings were mostly set up around a specific task e.g. discussing the solution of a human relation problem. The behavior of the group and its individuals towards problem evaluation and decisions were recorded, and categorized with the Bales Interaction Process Analysis (35, 36, 38). Thereby, the social interactions were found to be altered in a dose dependent manner. For small doses of LSD (25 - 50 µg) interaction was increased, whereas it leveled out on moderate doses (75 – 100 µg), and finally decreased on high doses up to 200 µg where subjects were less proactive in conversations (35-39). Changes in socio-emotional behavior were observed in all groups but with different outcomes (35-39). Alcoholics rose in positive emotional behavior whereas schizophrenics rose in positive as well as negative behaviors (36). Overall LSD was reported as an effective tool for increasing social interaction and gaining insight, making it thereby a useful therapeutic agent. Besides the psychological and socio-emotional effects, researchers were interested in the metabolism of LSD and its dose-relation to these effects.

For the determination of the metabolic fate, ^{14}C -labeled LSD was administered to animals, and measurements of radioactivity were used for the quantification, which was the method with the highest sensitivity and specificity during this research era (40-44). Experiments with ^{14}C labeled LSD in rats, mice, guinea pigs, and cats showed a rapid uptake in to the blood, distribution among the organs where LSD undergoes rapid chemical alteration, followed by a steady elimination in to the bile and the small intestine (40-44). Enterohepatic re-absorption was found to be negligible (44, 45). There was also a difference in metabolism across the various species. In rats, mice, guinea pigs, and cats, the biliary/faecal excretion dominated (40-44) whereas urinary excretion was dominant in rhesus monkeys (43). In rodents, the major metabolites in bile and urine were found to be 13- and 14-hydroxy-LSD glucuronides (43). In faeces the deconjugated forms, 13- and 14-hydroxy-LSD were dominating, probably cleaved by gut bacteria. In rhesus monkeys, 13- and 14-hydroxy-LSD accounted only for a minor part of the metabolites, but the major metabolites could not be clearly identified. However, the formation of an additional metabolite formed out of 2-oxo-LSD was described, and named "naphthostyryl compound" (43). This compound could be the precursor of the recently identified major human metabolite, 2-oxo-3-hydroxy-LSD (46). A further identified metabolite was de-ethyl-LSD, or lysergic-acid-monoethylamide (LAE). In vitro studies with liver microsomes additionally yielded nor-LSD as potential metabolite, however it could not be confirmed in-vivo (47). Out of the various LSD metabolites, 13-hydroxy-LSD and LAE were found to be active in animals (43).

In humans, the metabolism of LSD is largely unknown and was less investigated compared to the one in animals. The only two studies were done in the 1960s and 1970s. Single intravenous doses of 2 $\mu\text{g/kg}$ in five healthy male subjects, and single oral doses of 160 μg in 13 healthy male subjects were administered (48, 49). The only small pharmacokinetic study was done with the results from the study by Aghajanian et al. following the intravenous dose of 2 $\mu\text{g/kg}$ and they proposed a three-compartmental model (48, 50-52). Plasma concentrations were 6-7 ng/ml 30 min after intravenous administration, 4 - 6 ng/ml at 30 - 120 min, and approximately 1 ng/ml at 8 h. The elimination half-life of LSD was found to be 3 h (48, 50). This was also the first time that the effects of LSD, represented by a score of impairment in

solving a mathematical task, were linked to the plasma concentration (48, 50-52). The group of Upshall et al., which orally administered 160 µg of LSD, measured plasma concentrations in a fasted state, following a light breakfast, or a full breakfast. They observed a difference in plasma concentrations between men in a fasted state and men who had a full breakfast, suggesting, that the amount and composition of food has an effect on LSD plasma levels (49). The effects of two in-vivo identified metabolites were also investigated in humans. Intramuscular application of up to 1'200 µg LAE led to strong psychological effects, comparable to those after oral administration of 100 µg LSD (53). The effects were described faster in onset, but lasted only up to 2.5 hours. In contrast, oral administration of 300 µg 2-oxo-LSD did not induce any psychological effects (44).

Both human studies used fluorimetric assays for the measurements of their plasma samples. They made use of LSD's fluorescence and its UV-light catalyzed hydration to the non-fluorescent lumi-LSD (10-Hydroxy-9,10-dihydro-LSD) (48, 49). However, this method clearly lacked specificity (48, 49). Overall, human pharmacokinetic data is very sparse and new technologies such as LC-MS/MS allow to measure substance concentrations more precisely and also to further characterize metabolites. Indeed, more recent in-vitro studies using human liver microsomes and analysis of human urine samples have confirmed the presence of LAE, 2-oxo-LSD, 13- and 14-hydroxy-LSD, and further identified nor-LSD, lysergic-acid-ethyl-2-hydroxyethylamide (LEO), tri-oxo-LSD and 2-oxo-3-hydroxy-LSD as potential human metabolites (54, 55). However, systematic information about their presence after controlled intake is still missing.

Contrary to the unknown pharmacokinetic parameters, the pharmacodynamics, including subjective and autonomic effects, were widely investigated. The dose range for a typical LSD reaction was estimated to be 50 - 200 µg. A variety of different doses and routes of application have been used in different study populations including healthy subjects and patients (2, 14, 29). Therefore, descriptions of the psychological effects were varying and depended on the investigated study population, route of administration, dose of LSD, setting of the experiment, and expectations of subjects and investigators. Generally, symptoms could be classified among three characteristics: Somatic, perceptual, and psychologic effects.

In humans, LSD produces changes in perception, cognition, and emotions that last for up to 12 hours (9, 14, 23). Similar to other serotonergic drugs, mild or moderate anticipatory anxiety is common at the onset of the drug effect (56). During the time of full effect, mood changes are very frequent, mostly towards positive mood states (2, 14). Perceptual changes include illusions, pseudo-hallucinations, intensified color perception, synesthesia, and alterations in time perception (2, 14, 29). Alterations of thinking may include imaginative thoughts, broader and unusual associations, re-experiencing biographic memories, or mystical-type experiences (2, 14). Furthermore, LSD acutely impairs psychomotoric function including coordination and reaction time (2, 14, 29). Under controlled and supportive conditions, these phenomena are mostly experienced in a positive way and may have lasting positive effects on attitude and personality including greater appreciation of music, art, and nature, greater tolerance of others, and increased creativity and imagination (21). However, dysphoria, anxiety, and mild transient ideas of reference or paranoid thinking may also occur in some subjects. However, they are mostly attributable to uncontrolled conditions and can be readily managed with reassurance in a controlled setting (2, 14, 23, 29).

These numerous investigations prove that there is considerable previous experience with the use of LSD in humans, both with regard to research and clinical application. Psychotherapists have used LSD in thousands of patients and thus made LSD one of the most studied pharmacological substances with more than 4000 published reports (9, 14, 24).

These scientific activities came to a halt as a result of the political concerns in response to the increasing abuse of LSD starting in the end of the 1960s. Since the 1970s, clinical research using scheduled hallucinogenic substances like LSD has been prohibited in most countries, with only a few exceptions. From 1988 to 1993, LSD was legally used in Switzerland in LSD-assisted psychotherapy in 170 patients with a wide range of clinical disorders (57). Further uses of LSD were re-recognized and included its use in brain research (14), treatment of cluster headache (58, 59), alcoholism (60), and as an adjunct to psychotherapy (61). A first placebo-controlled pilot study using LSD in patients suffering from anxiety associated with advanced-stage life threatening diseases showed a potential therapeutic value (61).

Although some of the earlier research produced promising results, it became also clear that the initial studies conducted with LSD do not meet today's research standards. For instance, no optimal methodological procedures, e.g. double-blind, placebo-controlled studies, were used (14, 24). In addition, many of the techniques used today were not available or not as developed at that time. Specifically, comprehensive validated psychological test systems, sophisticated measures of physiological and endocrine parameters, neuroimaging or analytical techniques were unavailable or sparse. Hence, almost no scientific clinical pharmacological data on LSD is available.

2.3 The Future of LSD or Aims of the PhD Project

Despite very intensive research during the 1950s to 1970s, there are still a lot of research questions to be answered. First, most previous investigations do not meet our present scientific and ethical standards and have therefore to be replicated. The results from earlier studies were primarily observational and thus very subjective. Second, technological progress allows us to use new and more modern approaches such as imaging techniques. Third, LSD use is still very prevalent. Among young adults (15- to 34-year-olds), lifetime prevalence of LSD use varies from 0.1% to 5.4% in the EU (62) and up to 7% in the US population (63). Here in Basel, we registered over 13 cases with an acute LSD intoxication on the emergency department of the University Hospital Basel between October 2013 and September 2015 (64-66). Because of this renewed interest and the lack of state of the art human pharmacological data, we decided to conduct two placebo-controlled studies in healthy subjects.

We aimed to better characterize the pharmacology of LSD using sensitive and validated analytical and psychometric tools. One aim of our project was to develop and validate LC-MS/MS methods to characterize the single-dose kinetics of LSD and establish pharmacokinetic information which is important for the evaluation of clinical study findings such as subjective effects, autonomic effects, and functional magnetic resonance imaging results. Additionally, our methods were used to analyze samples of LSD emergency toxicological cases on the emergency department of the University Hospital Basel. The detailed analytical methods and the development/validation procedures are described in detail in the following publications 1 and 6 (65, 67). We also investigated the subjective effects, effects on mood, perception, emotion recognition and empathy using sensitive, validated psychometric tools. Investigation of the LSD effect on autonomic parameters included assessment of blood pressure, heart rate, body temperature, and pupil diameter.

Further, we aimed to define the neuronal correlates of the effects of LSD using *functional magnetic resonance imaging* (fMRI) techniques. Thereby, the studies also provided basic data for the understanding of the role of the serotonin 5-HT_{2A} receptor in the regulation of mood in general and on emotion recognition and empathy.

Our studies generated objective, high-quality scientific information on the effects of LSD in healthy subjects, data that cannot be obtained with observational studies. Overall, our placebo-controlled studies using LSD in healthy subjects were primarily descriptive in nature and with a focus on the tolerability and safety which is needed for future projects. For both clinical studies we used a double-blind placebo-controlled cross-over design with two treatment conditions (LSD and placebo). Thus, subjects served as their own controls omitting within-subject variability and markedly increased study power. The treatment order was counter-balanced with washout periods of at least 7 days between the test days. The placebo condition mainly served as a control condition for the subjective and somatic measures. Study 1 used a dose of 200 µg LSD and placebo in 16 subjects (8 men, 8 women), and Study 2 used 100 µg LSD and placebo in 24 subjects (12 men, 12 women). Detailed information about the inclusion and exclusion criteria for each study is explained in the following publications 3 and 4 (68-71). All data were obtained with the same psychometric questionnaires which were already used with other psychoactive and stimulant drugs in our group (72-74) and by others (12, 75-77). Detailed description of each test is part of the respective publications 3, 4, and 5 (69, 70, 78). Both studies were conducted in accordance with the Declaration of Helsinki and were approved by the local ethics committee. The administration of LSD to healthy subjects was authorized by the Swiss Federal Office for Public Health (BAG). The studies in the 1950s to 1970s have all used d-LSD tartrate (LSD-25, molecular weight 398), whereas we used d-LSD hydrate (molecular weight 323) what corresponds to a higher dose of LSD-25 (+23%).

3. Publications

3.1 Publication 1

Development and validation of a rapid turboflow LC-MS/MS method for the quantification of LSD and 2-oxo-3-hydroxy LSD in serum and urine samples of emergency toxicological cases

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Development and validation of a rapid turboflow LC-MS/MS method for the quantification of LSD and 2-oxo-3-hydroxy LSD in serum and urine samples of emergency toxicological cases

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Abstract Lysergic acid diethylamide (LSD) is a widely used recreational drug. The aim of the present study is to develop a quantitative turboflow LC-MS/MS method that can be used for rapid quantification of LSD and its main metabolite 2-oxo-3-hydroxy LSD (O-H-LSD) in serum and urine in emergency toxicological cases without time-consuming extraction steps. The method was developed on an ion-trap LC-MS/MS instrument coupled to a turbulent-flow extraction system. The validation data showed no significant matrix effects and no ion suppression has been observed in serum and urine. Mean intraday accuracy and precision for LSD were 101 and 6.84 %, in urine samples and 97.40 and 5.89 % in serum, respectively. For O-H-LSD, the respective values were 97.50 and 4.99 % in urine and 107 and 4.70 % in serum. Mean interday accuracy and precision for LSD were 100 and 8.26 % in urine and 101 and 6.56 % in serum, respectively. For O-H-LSD, the respective values were 101 and 8.11 % in urine and 99.8 and 8.35 % in serum, respectively. The lower limit of quantification for LSD was determined to be 0.1 ng/ml. LSD concentrations in serum were expected to be up to 8 ng/ml. 2-Oxo-3-hydroxy LSD concentrations in urine up to 250 ng/ml. The new method was accurate and precise in the range of expected serum and urine concentrations in patients with a suspected LSD intoxication. Until now, the method has been applied in five cases with suspected LSD intoxication where the intake of the drug has been verified four times with LSD concentrations in serum in the range of 1.80–14.70 ng/ml and

once with a LSD concentration of 1.25 ng/ml in urine. In serum of two patients, the O-H-LSD concentration was determined to be 0.99 and 0.45 ng/ml. In the urine of a third patient, the O-H-LSD concentration was 9.70 ng/ml.

Keywords LSD · O-H-LSD · LC-MS · Lysergic acid diethylamide · 2-Oxo-3-hydroxy LSD · Blood · Urine

Introduction

Lysergic acid diethylamide (LSD) is a psychoactive substance changing the state of consciousness and perception. Its psychedelic effects made it popular as a recreational drug, especially in the early 1970s, but still today LSD is widely used [1]. Additionally, LSD (200 µg) has also recently been used in a clinical study as adjunct to psychotherapy [2]. LSD is one of the most potent psychotropic drugs and is used in low doses. Typical recreational doses of LSD range from only 25 to 200 µg with long-lasting, dose-dependent psychotropic effects [1]. Hence, low blood and urine concentrations are posing a challenge to all analytical methods.

LSD can only be detected in blood up to 8 h after administration due to serum concentrations in the low nanogram per milliliter range. 2-Oxo-3-hydroxy LSD (O-H-LSD) is the main metabolite present in urine at concentrations 16–34 times higher than LSD [3, 4]. To our knowledge, O-H-LSD has only been detected once in blood in a postmortem case [5]. According to Li et al. and Klette et al. LSD and O-H-LSD were regarded as stable under storage conditions of –20 °C [6, 7].

Most published methods for LSD detection use either GC-MS or LC-MS/MS with a single-stage quadrupole [4, 5, 8–12]. The aim of the present study was to develop a turboflow LC-MS/MS method with the purpose of rapid quantification of LSD and its main metabolite in serum and

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urine in emergency toxicological cases without time-consuming extraction steps.

The method was developed using an ion-trap LC-MS/MS instrument in selected reaction monitoring (SRM) mode after atmospheric pressure ionization (APCI) for the quantification of LSD and O-H-LSD in urine and serum samples. Poch et al. used a similar APCI LC-MS/MS ion-trap instrument, but mainly for the detection of O-H-LSD [3].

Favretto et al. improved the method, but switched to electrospray ionization for suitable analysis of LSD and O-H-LSD in blood, urine, and vitreous humor [13]. Our method was established and successfully applied in five emergency toxicological cases with a suspected LSD intoxication. Additionally, the method will be used for the analysis of both blood and urine samples from a double-blind, placebo-controlled clinical trial.

Materials and methods

Chemicals and reagents

HPLC-grade purity acetonitrile, acetone, methanol, 2-propanol, formic acid, and acetic acid were all purchased from Merck (Darmstadt, Germany). Ammonium acetate and ammonium carbonate were obtained in HPLC grade from Merck (Darmstadt, Germany). Distilled water was obtained from an in-house installed purifier (ELGA, Bucks, UK).

Drug-free serum lyophilisate and urine negative control as blank matrices were obtained from Bio Rad Laboratories (Irvine, CA, USA). LSD and LSD- d_3 were obtained from Lipomed (Arlesheim, Switzerland) and 2-oxo-3-hydroxy LSD (O-H-LSD) from Cerilliant (Round Rock, TX, USA).

LC-MS analysis

Equipment

The sample extraction system (Transcend TLX1 HPLC, Thermo Scientific, Basel, Switzerland) consisted of a Thermo PAL autosampler and two Accela 600 pumps as eluting and loading pumps. The autosampler and the sample extraction system were all controlled by Aria software (version 1.6.3) from Thermo Scientific (Basel, Switzerland). A cyclone and a C18XL turboflow column (Thermo Scientific, Basel Switzerland) for extraction, and a 3 μ m Betasil Phenyl/Hexyl column (Thermo Scientific, Basel, Switzerland) for chromatographic separation were used.

The online extraction system was coupled to a LTQ XL mass spectrometer from Thermo Scientific (Basel, Switzerland) using atmospheric pressure ionization (APCI), due to its performance regarding matrix effects [14, 15].

For the instrument control, the corresponding software package consisting of LTQ (v.2.6) for ion detection, Xcalibur (v.2.1.0) for method programming, and LC-Quan (v.2.6.1) for quantification (all Thermo Scientific, Basel, Switzerland) was used.

LC method

The method was based on a previously published method [16]. Four mobile phases were used in gradient mode. For extraction, loading B consisted of 10 mM ammonium carbonate in water; eluting A was 5 mM ammonium acetate in water containing 0.10 % formic acid and eluting B 5 mM ammonium acetate in methanol containing 0.50 % formic acid, respectively.

Loading B was used as alkaline loading buffer, eluting A and B for chromatographic separation. Loading and Eluting C (acetonitrile/acetone/2-propanol, 1:1:1 (V/V/V)) were used to clean the extracting and the analytical columns.

The gradient system with a total run time of 12 min is depicted in Table 1. Under the following gradient conditions, LSD and LSD- d_3 showed a retention time of 7.63 min, while O-H-LSD had a retention time of 6.34 min.

MS conditions

For the quantification of LSD and its metabolite, APCI was used as the ionization source in positive ion mode. Discharge current and discharge voltage were set to 5 μ A and 4.2 kV, respectively. The vaporizer temperature was optimized to 452 °C whereas sheath and auxiliary gas provided best results with flow rates of 40 and 20 arbitrary units (AU). The capillary temperature was set to 275 °C.

LSD and O-H-LSD were quantified using single reaction monitoring (SRM) of the corresponding mass transitions (LSD m/z 324.6 \rightarrow 223.23, O-H-LSD m/z 356.33 \rightarrow 338.33, LSD- d_3 m/z 327.21 \rightarrow 226.2). The system was tuned and optimized for the detection of LSD.

Standard solutions

LSD and LSD- d_3 were bought as 1 mg/ml reference standards in acetonitrile, while O-H-LSD as 0.1 mg/ml reference standard in acetonitrile. Stock solutions in acetonitrile containing 100,000 ng/ml LSD, LSD- d_3 , or 10,000 ng/ml O-H-LSD, respectively, were prepared in duplicate and stored at -20 °C in order to have different sets for quality control (QC) and calibration samples, respectively. Working solutions of each analyte at 1000, 100, 10, and 1 ng/ml in water were used for the preparation of QC and calibration samples as well as for matrix and selectivity experiments.

Table 1 Detailed extraction and analytical separation steps of the liquid chromatography method

Time (min)	Extraction				Analytical separation			
	Flow (μl/min)	%A	%B	%C	Flow (μl/min)	%A	%B	%C
0.00	2	–	100	–	0.30	99	1	–
0.83	0.50	–	100	–	0.30	99	1	–
0.92	0.50	–	–	100	0.30	80	20	–
1.58	0.05	–	–	100	0.30	55	45	–
2.03	0.50	–	–	100	0.30	40	60	–
4.03	0.03	–	–	100	0.30	2	98	–
9.03	0.01	–	–	100	0.50	2	98	–
11.03	2.	–	–	100	0.50	2	98	–
11.37	2	–	–	100	0.50	–	–	100
11.70	2	–	100	–	0.50	–	100	–
12.20	2	–	100	–	0.50	99	1	–
12.53	2	–	100	–	0.30	99	1	–

Sample preparation

To 100 μl of serum, 100 μl acetonitrile for protein precipitation and 10 μl of a LSD-*d*₃ internal standard solution (100 ng/ml) were added. An identical volume of urine was diluted with 50 μl of an ammonium acetate buffer (50 mM, pH 4) and 10 μl of the internal standard solution. The samples were then vigorously vortexed, centrifuged for 10 min at 13,200g and the supernatant afterwards transferred into autosampler vials.

Calibration

Calibration curve in serum was realized by spiking serum samples with LSD and O-H-LSD to concentrations of 0.10, 0.25, 0.50, 0.75, 1, 2.50, 5, 7.50, and 10 ng/ml plus a blank (matrix only) and zero sample (matrix plus internal standard). The highest calibration point in serum was adopted from the maximum plasma concentration out of available i.v. kinetic data [17].

The calibration curve in urine was realized by spiking urine samples with O-H-LSD to concentrations of 1.50, 5, 10, 25, 50, 100, 125, 250, and 500 ng/ml. LSD concentrations were 0.10, 0.50, 1, 2, 5, 10, 12, 25, and 50 ng/ml, respectively. The highest calibrator in urine was adopted from published data containing various analyzed urine samples [4].

Both calibration curves were fitted linearly using a weighting factor ($1/x^2$).

In order to demonstrate accuracy and precision of the method, five QC's in urine and six QC's in serum were used with every run. The concentrations of the QC samples can be seen in Tables 2 and 3.

Selectivity

Following the FDA validation guidelines [18], six urine and six serum samples from different patients and healthy volunteers were collected and analyzed to establish selectivity and check for unwanted interferences within both matrices.

Matrix effects and recovery

Matrix effects, recovery, and process efficiency were measured and calculated according to Matuszewski et al. [19]. Matrix effects in urine and serum were calculated as ratio of the peak area before extraction and divided by the peak area after extraction. In contrast to Matuszewski et al., the extraction step consisted of simple protein precipitation as bypassing the extraction step on our ion-trap system was not possible. Six serum and six urine samples were spiked once with LSD and O-H-LSD before and after extraction. The peak areas of the spiked samples were then compared with the area of the spiked mobile phase. Urine samples were spiked to 25 ng/ml LSD resp. 250 ng/ml O-H-LSD, serum samples to 10 ng/ml each. Recovery values were calculated as areas of standards spiked before extraction divided by the areas of standards spiked after extraction. The process efficiency was also adopted from Matuszewski et al. and calculated as ratio between the area of the standard spiked before extraction, and the areas of the standard in neat solution.

Limit of quantification

Drug-free serum and urine samples were spiked with different concentrations of LSD and O-H-LSD for the determination of the lower limit of quantification (LLOQ). The parent substance and metabolite ratio was determined 1:1

Table 2 Intraday precision and accuracy data of LSD and 2-oxo-3-hydroxy LSD measured in serum and urine at different concentrations

	Weighed-in concentration [ng/ml]		Measured concentration [ng/ml]		Mean precision SD [%]		Mean accuracy \pm SD [%]	
	Serum	Urine	Serum	Urine	Serum ($n=6$)	Urine ($n=6$)	Serum ($n=6$)	Urine ($n=6$)
LSD	0.10	0.10	0.098 \pm 0.006	0.106 \pm 0.007	6.3	6.5	98.40 \pm 4.8	106 \pm 7.7
	0.40	0.25	0.38 \pm 0.03	0.28 \pm 0.03	6.6	12.3	96.20 \pm 6.5	112 \pm 13.1
	0.80	0.60	0.82 \pm 0.03	0.53 \pm 0.03	4.6	5.0	103 \pm 6.3	88.80 \pm 4.1
	4	3.30	3.92 \pm 0.22	3.32 \pm 0.20	5.7	6.1	97.80 \pm 4.7	101 \pm 7.0
	8	33	7.52 \pm 0.49	31.70 \pm 1.39	6.6	4.4	93.9 \pm 5.8	96.0 \pm 4.1
	10		9.53 \pm 0.53		5.5		95.3 \pm 5.7	
O-H-LSD	0.10	1.50	0.104 \pm 0.008	1.45 \pm 0.05	8.0	3.6	104 \pm 8.3	96.4 \pm 3.2
	0.40	2.50	0.44 \pm 0.02	2.20 \pm 0.16	3.8	7.2	110 \pm 4.2	88.20 \pm 6.5
	0.80	6	0.88 \pm 0.02	6.25 \pm 0.07	2.5	1.2	110 \pm 2.8	104 \pm 4.7
	4	33	4.04 \pm 0.38	33.90 \pm 2.5	9.5	7.3	101 \pm 9.6	103 \pm 8.2
	8	333	8.20 \pm 0.28	321 \pm 18	3.4	5.7	102 \pm 3.5	96.20 \pm 6.0
	10		11.29 \pm 0.11		0.9		113 \pm 1.1	

LSD lysergic acid diethylamide, O-H-LSD 2-oxo-3-hydroxy lysergic acid diethylamide

in serum and assumed 1:10 in urine samples [4]. The LLOQ concentrations had to give a response at least five times greater than the blank. Additionally, precision had to be <20 % and the accuracy between 80 and 120 % using at least five determinations per matrix and concentration.

Carryover

Carryover was determined by quantification of different blanks, running between patient samples, calibrations, and quality controls.

Reproducibility

According to the FDA guidelines, a minimum of five determinations per concentration are recommended for determination of precision and accuracy [18].

The reproducibility of quantification was determined by measuring serum ($n=6$) and urine ($n=5$) quality controls (QC) once on 1 day (intraday precision and accuracy) and on six different days (interday precision and accuracy). All values had to fulfill the criteria of a variation coefficient (CV) below 15 %, resp. below 20 % at the LLOQ and accuracy between 80 and 120 %. For serum, six quality controls from LLOQ to

Table 3 Interday precision and accuracy data of LSD and 2-oxo-3-hydroxy LSD measured in serum and urine at different concentrations

	Weighed-in concentration [ng/ml]		Measured concentration [ng/ml]		Mean precision SD [%]		Mean accuracy \pm SD [%]	
	Serum	Urine	Serum	Urine	Serum ($n=6$)	Urine ($n=6$)	Serum ($n=6$)	Urine ($n=6$)
LSD	0.10	0.10	0.11 \pm 0.01	0.10 \pm 0.02	4.60	15.00	110 \pm 5.10	104 \pm 14.60
	0.40	0.25	0.39 \pm 0.02	0.26 \pm 0.02	4.20	8.80	97.5 \pm 4.10	105 \pm 9.30
	0.80	0.60	0.82 \pm 0.07	0.55 \pm 0.02	8.50	4.00	103 \pm 8.70	91.1 \pm 3.60
	4	3.30	3.97 \pm 0.34	3.32 \pm 0.22	8.60	6.70	99.2 \pm 8.60	101 \pm 6.50
	8	33	7.41 \pm 0.59	32.8 \pm 2.3	7.90	6.90	92.7 \pm 7.30	99.3 \pm 6.30
	10		10.1 \pm 0.55		5.50		101 \pm 5.50	
O-H-LSD	0.10	1.50	0.10 \pm 0.08	1.58 \pm 0.19	8.10	12.50	105 \pm 8.43	105 \pm 13.10
	0.40	2.50	0.39 \pm 0.03	2.64 \pm 0.35	8.40	13.40	98.3 \pm 8.20	105 \pm 14.10
	0.80	6	0.79 \pm 0.08	5.56 \pm 0.16	9.80	3.00	98.5 \pm 9.70	92.6 \pm 2.70
	4	33	3.79 \pm 0.35	34.8 \pm 2.2	9.20	6.60	94.8 \pm 8.70	105 \pm 6.40
	8	333	8.14 \pm 0.58	327 \pm 16.8	7.20	5.10	102 \pm 7.30	98.3 \pm 5.00
	10	—	10.1 \pm 0.76		7.60	—	101 \pm 7.60	—

LSD lysergic acid diethylamide, O-H-LSD 2-oxo-3-hydroxy lysergic acid diethylamide

the highest calibrator (0.10, 0.40, 0.80, 4, 8, 10 ng/ml) were measured once a day. For validation in urine, five QCs from 1.5 to 333 ng/ml were used.

Results

Selectivity

None of the blank urine or serum samples showed any interference within the measured mass range and time frame.

Matrix effects and recovery

The matrix effects in urine were 138 % for LSD and 122 % for O-H-LSD. Recovery in urine was calculated to be 90.00 and 87.80 %, respectively. Process efficiency in urine was 124 % for LSD and 107 % for O-H-LSD. Serum showed higher matrix effects with 128 % for LSD and 78.70 % for O-H-LSD. Recovery in serum was 64.00 % for LSD and 32.00 %, for O-H-LSD. The process efficiencies in serum were calculated to be 128 % for LSD and 79 % for O-H-LSD. No ion suppression was found for LSD or O-H-LSD in serum and urine, but as mentioned by Johansen and Jensen [10] LSD- d_3 would correct for any ion suppression. In various negative samples, small LSD concentrations below the LLOQ could be identified which derived from the deuterated internal standard. Following these findings, LSD- d_3 was measured ten times at different concentrations. The working solution of the standard (100 ng/ml) contained 0.12 % undeuterated LSD. This impurity in the peak area of LSD was subtracted from all calibrators, quality controls, and unknown samples.

Lower limits of quantification

The lowest accurate and precisely measurable concentration was 0.10 ng/ml and thereby determined as LLOQ for LSD and O-H-LSD in serum. In urine samples, the LLOQ was determined at 0.10 ng/ml for LSD and 1.50 ng/ml for O-H-LSD.

Carryover

No carryover was found for LSD and O-H-LSD in serum samples. In contrast, a slight carryover (0.10 %) was found for O-H-LSD in urine samples following the highest QC (333 ng/ml) and the highest calibration (500 ng/ml) in urine. As a consequence, a second consecutive blank was inserted between and the carryover was reduced to 0.01 %.

Reproducibility

Calibration curves in urine were linear for both substances, LSD and O-H-LSD with R^2 greater than 0.98. Mean intraday accuracy and precision in serum were 97.40 resp. 5.89 % for LSD and 107 resp. 4.70 % for O-H-LSD (see Table 2). Mean interday accuracy and precision for LSD and O-H-LSD were 101 resp. 6.56 % and 99.80 resp. 8.35 %, respectively (see Table 3).

Linearity

LSD and O-H-LSD calibration curves in serum were linear over the range from 0.10 to 10 ng/ml with a mean correlation coefficient (R^2) of 99.86 %. The calibration curves of the mean values are shown in Fig. 1. Error bars indicate the standard error of the mean.

Calibration curves of LSD and O-H-LSD in urine were linear over the concentration range from 1.50 ng/ml to 333 ng/ml. R^2 was found to be 99.93 %. The detailed calibration curve is shown in Fig. 2.

Toxicological cases

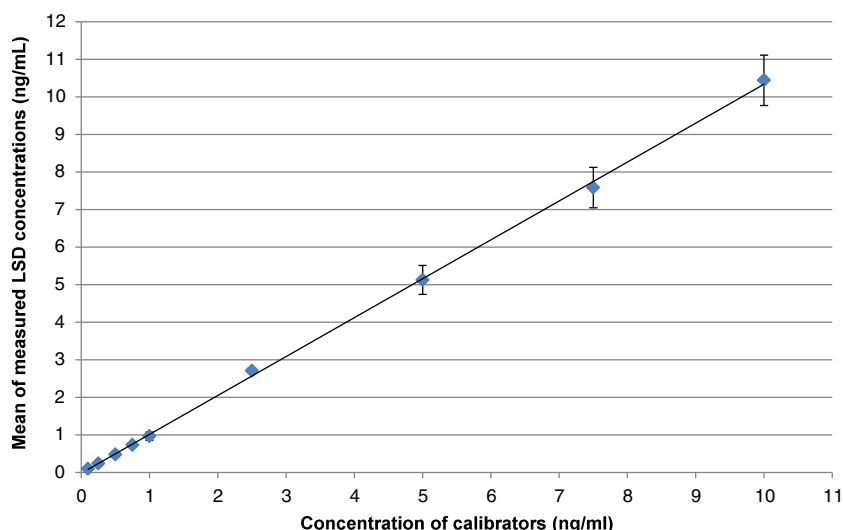
In the period from January to September 2014, five patients were admitted to the emergency department (ED) of the University Hospital Basel with suspected LSD intoxication. In all five cases, LSD consumption could be confirmed. Routinely, a LC-MS/MS method screening over 700 substances in serum was run to detect the intake of other medication and designer drugs.

As a summary, all in vivo measured concentrations in the matrices available from the emergency department can be found in Table 4.

Case 1

A 17-year-old girl was brought to the ED with acute confusion and loss of sense of time and orientation. She admitted consumption of two sugar cubes and one blot with LSD (estimated dose, 750 µg). A plasma sample for drug screening was taken approximately 3 h after ingestion. The patient was under chronic treatment with trazodone for depression. An additional LC-MS/MS screen in serum showed the presence of THC and trazodone. Quantification of LSD revealed a level of 14.70 ng/ml and a quantifiable O-H-LSD level of 0.99 ng/ml in serum. The only other published case where O-H-LSD could be detected in blood so far, was in a reanalyzed fatal case 10 years after collection [5]. Figure 3 shows the chromatogram of LSD, LSD- d_3 and O-H-LSD in the serum of this patient.

Fig. 1 Shows the mean calibration curve of LSD in serum from the validation measurements. The determination coefficient was 0.9995



Case 2

A 17-year-old male was brought by the ambulance to the ED with thoracic pressure, restlessness, and dyspnea. He admitted the intake of one sugar cube with LSD (estimated dose 250 µg) at 8 p.m. with concomitant consumption of cannabis. He reported onset of the symptoms at 10 p.m., 2 h post-consumption. In the emergency department, the patient was treated with lorazepam and acetaminophen. Serum analysis revealed a LSD concentration of 1.80 ng/ml in a blood sample taken at 11 p.m.

Case 3

A 21-year-old male was admitted to the ED by ambulance and the police because of aggressive and uncooperative behavior after consumption of an alleged LSD blot. No information

about the time-point of the LSD ingestion was available from anamnesis. Serum analysis showed an LSD concentration of 6.10 ng/ml and an O-H-LSD concentration of 0.45 ng/ml. An additional LC-MS/MS screening revealed the presence of THC, cocaine, and amphetamine.

Case 4

A 45-year-old male presented himself to the ED with agitation, disorientation, and intense visual hallucinations. He was partying for 2 days and consumed alcohol, LSD, cocaine, and cannabis. The time-point of the LSD intake was not reported. The LC-MS/MS screening confirmed the intake of THC and cocaine. Quantification of the serum LSD level detected 4.10 ng/ml LSD, but no quantifiable O-H-LSD.

Fig. 2 Shows the mean calibration curve of 2-oxo-3-hydroxy LSD in urine from the validation measurements. The determination coefficient was 0.9993

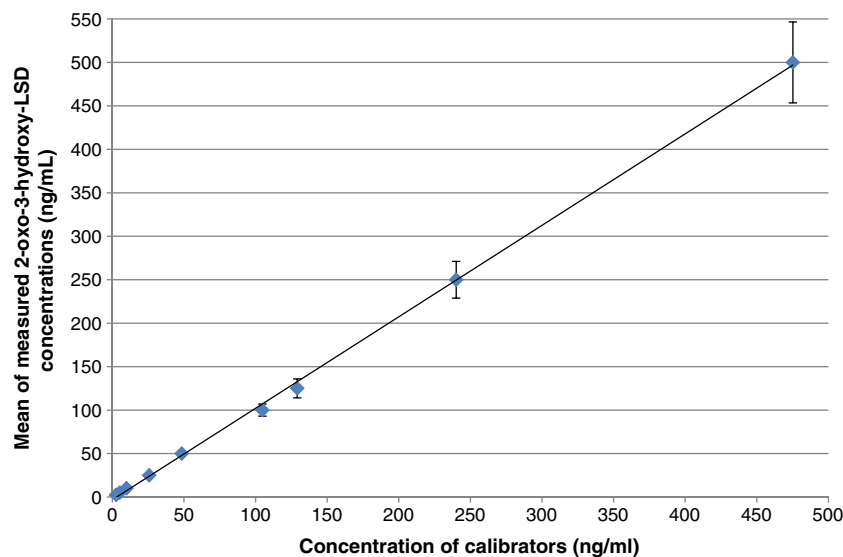


Table 4 Measured concentration of LSD and 2-oxo-3-hydroxy LSD in serum and/or urine in different patients

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Serum LSD	14.70 ng/ml	1.80 ng/ml	6.10 ng/ml	4.10 ng/ml	N/A
Serum O-H-LSD	0.99 ng/ml	<LLOQ	0.45 ng/ml	<LLOQ	N/A
Urine LSD	N/A	N/A	N/A	N/A	1.30 ng/ml
Urine O-H-LSD	N/A	N/A	N/A	N/A	9.70 ng/ml

N/A matrix was not available from the emergency department; <LLOQ value was below the lower limit of quantification

Case 5

A 36-year-old male presented himself to the ED with tactile and visual hallucinations after consumption of an alcoholic beverage in a club. He suspected someone to have mixed some drugs in his drink. A screening for LSD in urine revealed 1.30 ng/ml LSD and 9.70 ng/ml O-H-LSD, respectively. An additional LC-MS/MS screening in urine confirmed the presence of THC. No time-point of the drink consumption or start of the LSD effect was reported.

Discussion and conclusion

The development of a sensitive method for the measurement of LSD and its metabolite is an analytical challenge due to its low concentrations in serum and urine.

Purification procedures with solid-phase or liquid-liquid extraction can certainly lead to better sensitivity of the LC-MS/MS method, but form a time-consuming procedure [5]. The short run time of 12 min was mainly given by retention times of LSD, LSD-*d*₃, and O-H-LSD. The additional time following the LSD and LSD-*d*₃ peak was necessary to ensure

clean peak separation and flushing the columns to minimize carryover.

Our purpose was to establish a fast and reliable method for application in emergency toxicological cases where time is crucial. With a short method run of 12 min and minimum sample preparation, results will be more quickly available so that a fast diagnosis is possible. The method was applied in five toxicology cases where consumption of LSD could be confirmed four times in serum and once in urine.

Due to the fast method and obviation of purification steps, a slight loss in sensitivity was accepted. LLOQ and LOD in serum were hence higher than in other comparable methods [5, 8–10, 13]. Some showed LOQ's as low as 0.02 ng/ml for LSD but needed sample preparation and a longer run time [5]. In contrast, our method was mainly developed to rapidly detect levels of LSD that occur during acute intoxication. The range of expected LSD concentrations in serum was difficult to determine because only few pharmacokinetic data is available. In fact, only one pharmacokinetic study with controlled administration of LSD exists. In this study, peak plasma concentrations of LSD were 4–6 ng/ml 1–2 h after administration of LSD (intravenously at 2 µg/kg) [17]. Therefore, we chose 10 ng/ml as highest calibrator to cover typically used oral doses of LSD (100–400 µg) [1]. However, one case

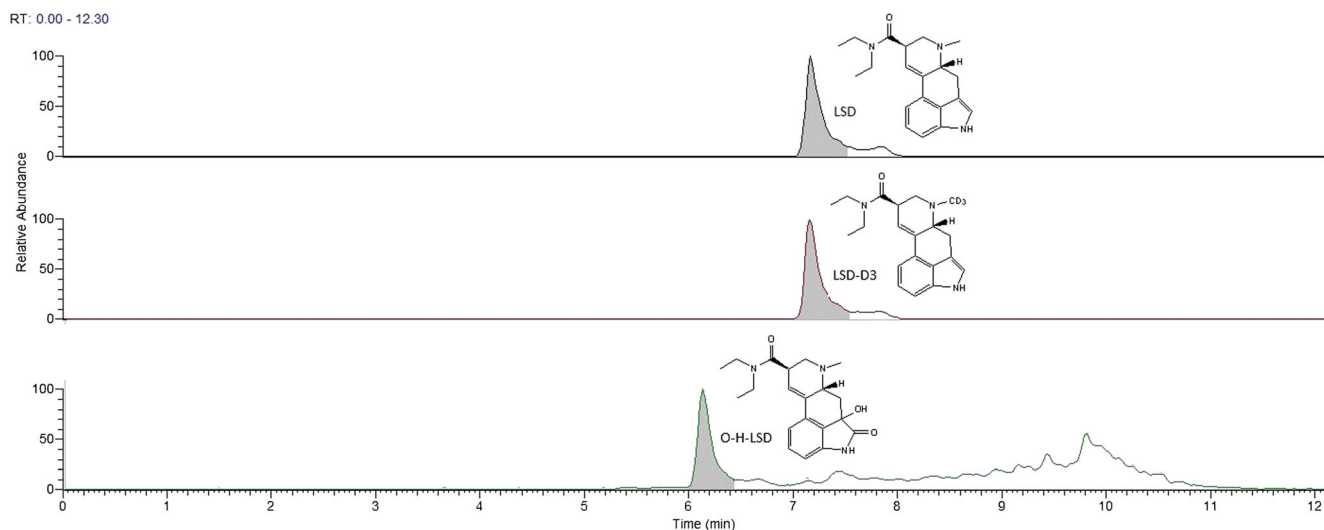


Fig. 3 Chromatogram and the respective structural formulas of LSD, LSD-*d*₃, and 2-oxo-3-hydroxy LSD in the serum sample of patient 1

was found with a LSD concentration of 14 ng/ml in plasma among the intoxication cases presented here.

This sample had to be diluted (1:1 with distilled water) in order to determine the correct result. Expected urine concentrations and the calibration range were established considering already published data [3, 4]. Our method fulfilled all criteria for measurement of emergency toxicological cases. All four cases showed concentrations of LSD in serum in the range of 1.80–14.70 ng/ml. Additionally, to our knowledge, for the first time, we describe the quantification of O-H-LSD in two patients in a concentration well above the LLOQ of our method.

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References

1. Passie T, Halpern JH, Stichtenoth DO, Emrich HM, Hintzen A (2008) The pharmacology of lysergic acid diethylamide: a review. *CNS Neurosci Ther* 14(4):295–314
2. Gasser P, Holstein D, Michel Y, Doblin R, Yazar-Klosinski B, Passie T, Brenneisen R (2014) Safety and efficacy of lysergic acid diethylamide-assisted psychotherapy for anxiety associated with life-threatening diseases. *J Nerv Ment Dis* 202(7):513–520
3. Poch GK, Klette KL, Anderson C (2000) The quantitation of 2-oxo-3-hydroxy lysergic acid diethylamide (O-H-LSD) in human urine specimens, a metabolite of LSD: comparative analysis using liquid chromatography-selected ion monitoring mass spectrometry and liquid chromatography-ion trap mass spectrometry. *J Anal Toxicol* 24(3):170–179
4. Poch GK, Klette KL, Hallare DA, Manglicmot MG, Czarny RJ, McWhorter LK, Anderson CJ (1999) Detection of metabolites of lysergic acid diethylamide (LSD) in human urine specimens: 2-oxo-3-hydroxy-LSD, a prevalent metabolite of LSD. *J Chromatogr B Biomed Sci Appl* 724(1):23–33
5. Chung A, Hudson J, McKay G (2009) Validated ultra-performance liquid chromatography-tandem mass spectrometry method for analyzing LSD, iso-LSD, nor-LSD, and O-H-LSD in blood and urine. *J Anal Toxicol* 33(5):253–259
6. Klette KL, Horn CK, Stout PR, Anderson CJ (2002) LC-MS analysis of human urine specimens for 2-oxo-3-hydroxy LSD: method validation for potential interferants and stability study of 2-oxo-3-hydroxy LSD under various storage conditions. *J Anal Toxicol* 26(4):193–200
7. Li Z, McNally AJ, Wang H, Salamone SJ (1998) Stability study of LSD under various storage conditions. *J Anal Toxicol* 22(6):520–525
8. Berg T, Jorgenrud B, Strand DH (2013) Determination of buprenorphine, fentanyl and LSD in whole blood by UPLC-MS-MS. *J Anal Toxicol* 37(3):159–165
9. Canezin J, Cailleux A, Turcant A, Le Bouil A, Harry P, Allain P (2001) Determination of LSD and its metabolites in human biological fluids by high-performance liquid chromatography with electrospray tandem mass spectrometry. *J Chromatogr B Biomed Sci Appl* 765(1):15–27
10. Johansen SS, Jensen JL (2005) Liquid chromatography-tandem mass spectrometry determination of LSD, ISO-LSD, and the main metabolite 2-oxo-3-hydroxy-LSD in forensic samples and application in a forensic case. *J Chromatogr B Anal Technol Biomed Life Sci* 825(1):21–28
11. Musshoff F, Daldrup T (1997) Gas chromatographic/mass spectrometric determination of lysergic acid diethylamide (LSD) in serum samples. *Forensic Sci Int* 88(2):133–140
12. Nelson CC, Foltz RL (1992) Determination of lysergic acid diethylamide (LSD), iso-LSD, and N-demethyl-LSD in body fluids by gas chromatography/tandem mass spectrometry. *Anal Chem* 64(14):1578–1585
13. Favretto D, Frison G, Maietti S, Ferrara SD (2007) LC-ESI-MS/MS on an ion trap for the determination of LSD, iso-LSD, nor-LSD and 2-oxo-3-hydroxy-LSD in blood, urine and vitreous humor. *Int J Legal Med* 121(4):259–265
14. Caban M, Migowska N, Stepnowski P, Kwiatkowski M, Kumirska J (2012) Matrix effects and recovery calculations in analyses of pharmaceuticals based on the determination of β -blockers and β -agonists in environmental samples. *J Chromatogr A* 1258:117–127
15. Dams R, Huestis MA, Lambert WE, Murphy CM (2003) Matrix effect in bio-analysis of illicit drugs with LC-MS/MS: influence of ionization type, sample preparation, and biofluid. *J Am Soc Mass Spectrom* 14(11):1290–1294
16. Mueller DM, Duretz B, Espourteille FA, Rentsch KM (2011) Development of a fully automated toxicological LC-MS/MS screening system in urine using online extraction with turbulent flow chromatography. *Anal Bioanal Chem* 400(1):89–100
17. Aghajanian GK, Bing OH (1964) Persistence of lysergic acid diethylamide in plasma of human subjects. *Clin Pharmacol Ther* 5:611–614
18. Service USDoHaH (2001) Guidance for the industry—bioanalytical method validation
19. Matuszewski BK, Constanzer ML, Chavez-Eng CM (2003) Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. *Anal Chem* 75(13):3019–3030

3.2 Publication 2

Pharmacokinetics and Concentration-Effect Relationship of Oral LSD in Humans

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RESEARCH ARTICLE

Pharmacokinetics and Concentration-Effect Relationship of Oral LSD in Humans

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Abstract

Background: The pharmacokinetics of oral lysergic acid diethylamide are unknown despite its common recreational use and renewed interest in its use in psychiatric research and practice.

Methods: We characterized the pharmacokinetic profile, pharmacokinetic-pharmacodynamic relationship, and urine recovery of lysergic acid diethylamide and its main metabolite after administration of a single oral dose of lysergic acid diethylamide (200 µg) in 8 male and 8 female healthy subjects.

Results: Plasma lysergic acid diethylamide concentrations were quantifiable (>0.1 ng/mL) in all the subjects up to 12 hours after administration. Maximal concentrations of lysergic acid diethylamide (mean \pm SD: 4.5 ± 1.4 ng/mL) were reached (median, range) 1.5 (0.5–4) hours after administration. Concentrations then decreased following first-order kinetics with a half-life of 3.6 ± 0.9 hours up to 12 hours and slower elimination thereafter with a terminal half-life of 8.9 ± 5.9 hours. One percent of the orally administered lysergic acid diethylamide was eliminated in urine as lysergic acid diethylamide, and 13% was eliminated as 2-oxo-3-hydroxy-lysergic acid diethylamide within 24 hours. No sex differences were observed in the pharmacokinetic profiles of lysergic acid diethylamide. The acute subjective and sympathomimetic responses to lysergic acid diethylamide lasted up to 12 hours and were closely associated with the concentrations in plasma over time and exhibited no acute tolerance.

Conclusions: These first data on the pharmacokinetics and concentration-effect relationship of oral lysergic acid diethylamide are relevant for further clinical studies and serve as a reference for the assessment of intoxication with lysergic acid diethylamide.

Keywords: LSD, O-H-LSD, pharmacokinetics, pharmacodynamics, plasma, urine

Trial registration: Registration identification number: NCT01878942

ClinicalTrials.gov: <http://clinicaltrials.gov/ct2/show/NCT01878942>.

Introduction

Lysergic acid diethylamide (LSD) is a prototypical hallucinogen (Nichols, 2004; Passie et al., 2008). LSD became famous as a psychedelic in the 1960s, and its recreational use continues (Passie et al.,

2008). However, no clinical research has been conducted with LSD since the 1970s until recently (Gasser et al., 2014; Kupferschmidt, 2014). Almost no scientific clinical pharmacological data on LSD

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are available. Specifically, the pharmacokinetics (PK) of oral LSD in humans are unknown. A small PK study administered single intravenous doses of 2 µg/kg in 5 healthy male human subjects (Aghajanian and Bing, 1964). Blood samples were taken up to 8 hours after administration. Plasma concentrations were 6 to 7 ng/mL 30 minutes after intravenous administration, 4–6 ng/mL at 30–120 min, and approximately 1 ng/mL at 8 hours. The mean plasma elimination half-life of LSD was estimated at 175 minutes in this previous study. In another study, single oral doses of 160 µg were administered to 13 male human subjects, and blood was sampled nonsystematically at various time points up to a maximum of 2.5 to 5 hours. Plasma levels peaked 40 to 130 minutes after LSD administration, and peaks ranged from 1.8 to 8.8 ng/mL (Upshall and Wailling, 1972). The dataset and short sampling time did not allow the calculation of PK parameters.

The aim of the present study was to characterize the single-dose kinetics and PK-pharmacodynamic relationships of LSD in healthy male and female subjects. For clinical and forensic toxicologists, it is important to know the toxicokinetics of LSD and how plasma concentrations of LSD are linked to its dynamic effects and signs of intoxication.

LSD was administered in a single oral dose of 200 µg. The same dose was used in a clinical study (Gasser et al., 2014). The dose used was within the range of doses (50–400 µg) taken for recreational purposes and expected to induce a full “LSD reaction” (Nichols, 2004; Passie et al., 2008). The study also evaluated the acute subjective, autonomic, and endocrine effects of LSD. The pharmacodynamics are reported in detail elsewhere (Schmid et al., 2014), but the PK-pharmacodynamic relationships are presented herein.

Methods

Study Design

The study used a double-blind, placebo-controlled, cross-over design with 2 experimental test sessions in balanced order. The washout periods between sessions were at least 7 days. The study was conducted in accordance with the Declaration of Helsinki and International Conference on Harmonization Guidelines in Good Clinical Practice and approved by the Ethics Committee of the Canton of Basel, Switzerland and the Swiss Agency for Therapeutic Products (Swissmedic). The administration of LSD to healthy subjects was authorized by the Swiss Federal Office for Public Health, Bern, Switzerland. The study was registered at ClinicalTrials.gov (NCT01878942). All of the subjects provided written informed consent after being given written and oral descriptions of the study, the procedures involved, and the effects and possible risks of LSD administration.

Participants

Sixteen healthy subjects (8 men and 8 women; mean age ± SD: 28.6 ± 6.2 years; range: 25–51 years) were included. The exclusion criteria are reported in detail elsewhere (Schmid et al., 2014) and included age <25 or >65 years, pregnancy, personal or family (first-degree relative) history of psychotic or major affective disorder, regular use of medications, chronic or acute physical illness, lifetime prevalence of illicit drug use >10 times (except for tetrahydrocannabinol), illicit drug use within the last 2 months, and illicit drug use during the study. Nine subjects were hallucinogen-naïve, and the other 7 had limited prior experience with hallucinogenic drugs, including 1 subject who had used LSD

once and 2 subjects who had used LSD twice. The subjects were asked to abstain from excessive alcohol consumption between test sessions and particularly limit their use to 1 drink on the day before the test sessions. Additionally, the participants were not allowed to drink xanthine-containing liquids after midnight before the study day. Three subjects were light smokers (<10 cigarettes/d) and were told to maintain their usual smoking habits but not smoke during the sessions. We performed urine drug tests at screening and before each test session using TRIAGE 8 (Biosite, San Diego, CA). No alcohol test was performed.

Study Outline

The test sessions began at 8:15 AM. A urine sample was taken to verify abstinence from drugs of abuse, and a pregnancy test was performed in women. An indwelling intravenous catheter was placed in an antecubital vein for blood sampling, and the subjects completed baseline measurements. LSD (200 µg) or placebo was administered at 9:00 AM. A standardized lunch and dinner was served at 1:30 PM and 5:30 PM, respectively. The subjects were sent home the next day at 9:30 AM after the 24-hour blood sample collection.

Drugs

Gelatin capsules that contained 100 µg LSD (D-LSD hydrate with a purity (high-performance liquid chromatography) >99%; Lipomed AG, Arlesheim, Switzerland), and corresponding placebo capsules were prepared with authorization from the Swiss Federal Office for Public Health. LSD was administered in a single absolute dose of 200 µg, corresponding to a dose of 2.84 ± 0.5 µg/kg body weight (mean ± SD; range: 2.04–3.85 µg).

Blood and Urine Sampling

Blood was collected into lithium heparin tubes 1 hour before and 0.5, 1, 1.5, 2.5, 3, 4, 6, 8, 10, 12, 16, and 24 hours after LSD administration. Urine (entire volume) was collected during 3 sampling periods: 0 to 8, 8 to 16, and 16 to 24 hours after LSD administration. Blood samples were immediately centrifuged, and plasma and urine were rapidly stored at -20°C until analysis within 2 to 6 months. Long-term stability (6 months) has been shown for LSD and 2-oxo-3-hydroxy-LSD (O-H-LSD) when kept under refrigerated or frozen conditions (Klette et al., 2002; Martin et al., 2013). The recovery (ng) of LSD and O-H-LSD was determined by multiplying the analyte urine concentrations (ng/mL) with the urinary volume (mL) of the respective sampling interval.

Analysis of LSD and O-H-LSD

LSD and O-H-LSD concentrations in plasma and urine were determined using a validated liquid-chromatography-tandem mass-spectrometry method as reported in detail in the [supplementary Material](#) online and elsewhere (Dolder et al., 2015). The lower limit of quantification was 0.1 ng/mL, and the upper limit of quantification was 10 ng/mL for LSD and O-H-LSD in both plasma and urine.

PK

The plasma concentration data were analyzed using noncompartmental methods using Phoenix WinNonlin 6.4 (Certara, Princeton, NJ). C_{max} and T_{max} values were obtained directly from

the observed data. The area under the concentration-time curve (AUC) from 0 to 24 hours after dosing (AUC_{24}) was calculated using the linear-up log-down trapezoidal method. The terminal elimination rate constant (λ_z) for LSD was estimated by log-linear regression after semilogarithmic transformation of the data using at least the last 3 data points of the terminal linear phase of the concentration-time curve. The terminal half-life was calculated using λ_z and the equation $t_{1/2} = \ln_2/\lambda_z$. The AUC to infinity was then determined by extrapolation of the AUC_{24} using λ_z . We also determined a separate half-life for the T_{max} to 12 hour interval, because the rate of elimination changed at 12 hours in many subjects (see [supplementary Figure S1](#) for all plots), and the decrease in plasma concentrations followed first-order kinetics in all subjects from T_{max} to 12 hours. For this phase, we estimated the elimination rate constant (λ) for LSD using at least 3 data points of the concentration-time curve. Thus, this half-life does not describe the slower decrease in the concentration of LSD observed in a subset of subjects beyond 12 hours or 16 hours. Individual concentration-time curves show that a slower terminal decrease in LSD concentrations occurred only beyond 12 hours (after eating dinner and during the night) and not concentration-dependent (ie, was not observed below a certain threshold concentration of LSD; see [supplementary Figure S1](#)). Renal clearance (mL/h) was calculated as urinary recovery_{24 urine} (ng)/ AUC_{24} (ng·h/mL).

Statistical Analyses

The analysis of the pharmacokinetic parameters was descriptive, and geometric means and 90% CIs are shown to account for nonnormally distributed data. The study included 8 subjects of each sex; the data are also presented for male and female subjects separately. However, the study was not sufficiently powered (power: 52%) to exclude sex differences in the PK of LSD (PASS Power Analysis, Kaysville, UT).

The primary pharmacodynamic study results were reported elsewhere ([Schmid et al., 2014](#)). The a priori hypothesis relating to the PK-pharmacodynamics as defined in the study protocol was that the pharmacodynamic effects of LSD would show no acute pharmacological tolerance (ie, no clockwise hysteresis in the concentration-effect relationship). To assess PK-pharmacodynamic relationships, the LSD-induced effect was determined as a difference from placebo in the same subject at the corresponding time point to control for circadian changes ([Schmid et al., 2014](#)). The pharmacodynamic changes after LSD administration for each time point were plotted against the respective plasma concentrations of LSD and graphed as hysteresis curves for each subject. Because pupil size measurements were unavailable at the same time points as plasma levels, pupil size values at 7 and 11 hours were matched with concentrations at 8 and 12 hours. No pupil size measurement was available for the 24-hour time point; therefore, we used the baseline value at $t = 0$ hours, assuming a return to baseline by 24 hours. The area within the hysteresis (A_H) was calculated as $AUC_{C0-C_{max}} - AUC_{C_{max}-C_{24}}$ using the trapezoidal rule. $A_H < 0$ indicates counter-clockwise hysteresis (lag time between concentration and effect due to absorption/distribution processes). $A_H > 0$ indicates clockwise hysteresis (tolerance).

To estimate the plasma concentration of LSD at which 50% of the maximal response to LSD is reached (EC_{50}), a sigmoidal concentration-response (variable slope) model was fitted to the plasma concentration-effect data: $E = (E_{max} \times C_p^h) / (C_p^h + EC_{50}^h)$, in which E is the observed effect, C_p is the plasma LSD

concentration, E_{max} is the maximal effect, and h is the Hill slope using WinNonlin. Because of the hysteresis observed for most plasma-concentration effect curves, an indirect descriptive link model would be needed in which the plasma concentrations are linked to the pharmacodynamic parameter by an effect compartment, providing an estimate of the equilibration half-life between plasma and the effect compartment. However, because insufficient data pairs for the absorption phase ($0-C_{max}$) were available, we directly linked dynamic effects to the plasma concentrations using only data from C_{max} up to 24 hours after drug administration for this analysis. Statistical analyses were conducted using NCSS 2004 software (Statistical Software, Kaysville, UT).

Pharmacodynamic Measurements

Pharmacodynamic measures were included in this study to evaluate PK-pharmacodynamic relationships. Subjective effects were assessed repeatedly over time using visual analog scales (VASs) ([Hysek et al., 2014](#)), including “any drug effect,” “good drug effect,” and “bad drug effect.” The VASs were presented as 100-mm horizontal lines marked with “not at all” on the left and “extremely” on the right. The VASs were administered 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 16, and 24 hours after drug administration. Vital signs, including blood pressure, heart rate, and body (tympanic) temperature, were assessed repeatedly 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 hours after drug administration using previously reported methods ([Hysek et al., 2014](#)). Additionally, pupil size (dark-adapted maximal pupil diameter) was measured 1, 2.5, 4, 7, and 11 hours after drug administration using an infrared pupillometer (PRL-200, NeuroOptics, Irvine, CA) under standardized dark-light conditions as previously reported ([Hysek and Liechti, 2012](#)).

Results

PK

[Figure 1](#) shows the plasma-concentration-time curves for LSD and O-H-LSD. The PK parameters are shown in [Table 1](#). The plasma concentrations of LSD (>0.1 ng/mL) could be measured in all of the subjects up to 12 hours, in 14 subjects up to 16 hours, and in 11 subjects up to 24 hours after administration. Concentrations of LSD decreased following first-order kinetics up to 12 hours with a half-life of 3.6 ± 0.9 hours ([Figure 1b](#)). In some subjects, a slower decrease in plasma concentrations was observed late in time between 12 and 24 hours. This slower decrease occurred after the subjective effects of LSD had mostly subsided and the individual concentration-time curves showed that the slower decrease was dependent on time >12 hours (after eating dinner and during the night) and not on concentration (ie, below a certain concentration of LSD) (see [supplementary Figure S1](#)). The terminal half-life was 8.9 ± 5.9 hours including 4 subjects (S4-S7, see [supplementary Figure S1](#)) in whom concentrations of LSD at 24 hours showed no further decrease compared with the 16-hour concentrations.

The O-H-LSD concentration-time profiles could be determined for only 8 subjects, because metabolite concentrations were not present or fell below the lower limit of quantification in one-half of the subjects ([Figure 1c-d](#)). We could not show a difference in the pharmacokinetic profiles of LSD between male and female subjects ([Table 1](#)). The concentrations of LSD and O-H-LSD in urine and the urine recovery of LSD and O-H-LSD are shown in [Table 2](#). The mean molar concentrations of O-H-LSD (molecular

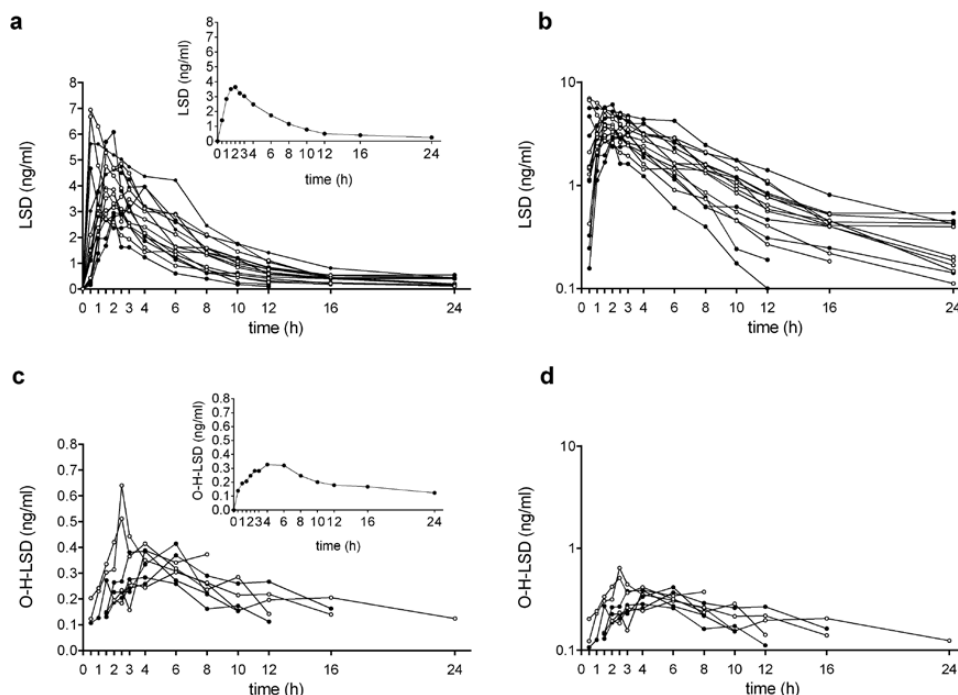


Figure 1. Pharmacokinetics (PK) of lysergic acid diethylamide (LSD) and 2-oxo-3-hydroxy-LSD (O-H-LSD). (a) Individual LSD plasma concentration-time curves with the geometric mean shown in the inset. Filled circles indicate male subjects, and open circles indicate female subjects. (b) Semilogarithmic plot of the individual concentrations of LSD. Curves are shown separately for each individual in the [supplementary Material \(supplementary Figure S1\)](#). First-order kinetics were observed in all 16 subjects up to 12 hours. LSD levels fell below the lower limit of quantification (0.1 ng/mL) in 2 subjects at 16 hours and 5 subjects at 24 hours. Slower elimination was observed between 12 and 24 hours. (c) Individual O-H-LSD plasma concentration-time curves in 8 subjects in whom metabolite concentrations could be determined, with the geometric mean shown in the inset. (d) Semilogarithmic plot of the individual concentrations of O-H-LSD. Curves are shown separately in [supplementary Figure S2](#). LSD was administered at $t = 0$ hours.

Table 1. Pharmacokinetic Parameters for LSD and O-H-LSD

			C_{\max} (ng/mL)	t_{\max} (h)	$t_{1/2}$ (h)	$t_{1/2}$ (h)	AUC_{24} (ng·h/mL)	AUC_{∞} (ng·h/mL)	CL_R (mL/min)
		N=	Geometric Mean (95%CI)	Median (range)	$T_{\max} -12$ h Mean \pm SD	Terminal Mean \pm SD	Geometric Mean (90%CI)	Geometric Mean (90%CI)	Mean \pm SD
LSD	All	16	4.3 (3.8–4.9)	1.5 (0.5–4)	3.6 ± 0.9	8.9 ± 5.9	26 (22–30)	28 (24–33)	79 ± 36
LSD	Male	8	4.4 (3.6–5.3)	1.5 (0.5–4)	3.5 ± 1.0	10.2 ± 6.7	25 (18–35)	28 (20–38)	88 ± 36
LSD	Female	8	4.2 (3.4–5.3)	1.5 (0.5–3)	3.8 ± 0.8	7.6 ± 5.1	26 (23–30)	28 (25–32)	71 ± 36
O-H-LSD	All	8	0.4 (0.3–0.5)	4 (2.5–6)			3.4 (2.6–4.3)	3.8 (2.8–5.3)	

Abbreviations: AUC, area under the plasma concentration-time curve; AUC_{∞} , AUC from time zero to infinity; AUC_{24} , from time 0–24; CL_R , renal clearance; C_{\max} , maximum observed plasma concentration; $T_{1/2}$, plasma half-life; T_{\max} , time to reach C_{\max} ; *O-H-LSD levels were above the limit of detection in only 8 subjects).

Table 2. Urinary Elimination of LSD and O-H-LSD

		LSD				O-H-LSD			
	N=	0–8 hours	8–16 hours	16–24 hours	0–24 hours	0–8 hours	8–16 hours	16–24 hours	0–24 hours
Urinary concentrations (ng/mL)									
all	16	0.96 ± 0.8	1.1 ± 1.8	0.70 ± 0.6		8.3 ± 4.7	17.7 ± 11	14.4 ± 10	
male	8	0.78 ± 0.4	0.82 ± 0.2	0.66 ± 0.6		6.6 ± 3.4	22.7 ± 14	11.2 ± 7	
female	8	1.1 ± 1.0	1.5 ± 2.6	0.74 ± 0.7		9.9 ± 5.4	12.7 ± 5.3	17.6 ± 12	
Urinary volume (L)									
all	16	1.4 ± 0.7	0.79 ± 0.4	0.47 ± 0.3					
male	8	1.8 ± 0.8	0.86 ± 0.5	0.63 ± 0.2					
female	8	1.1 ± 0.5	0.71 ± 0.4	0.30 ± 0.2					
Urinary recovery (nM) A_{e0-24}									
all	16	3.6 ± 2.6	2.0 ± 1.7	0.82 ± 0.5	6.4 ± 2.9	28.3 ± 15	35.9 ± 27	15.3 ± 7.8	79.5 ± 41
male	8	3.8 ± 2.4	2.0 ± 0.8	1.1 ± 0.6	6.8 ± 2.6	29.3 ± 19	49.6 ± 33	17.1 ± 8.6	96.1 ± 51
female	8	3.5 ± 2.8	2.0 ± 2.3	0.58 ± 0.3	6.0 ± 3.3	27.3 ± 13	$22.3 \pm 9^*$	13.4 ± 6.8	62.9 ± 18

Abbreviations: A_e , amount eliminated in nM; LSD, lysergic acid diethylamide; O-H-LSD, 2-oxo-3-hydroxy-LSD.

*Significant difference from men ($P < .05$). Values are mean \pm SD.

weight: 355.4) were 23.2, 49.9, and 40.6 pM/mL and 8, 14, and 19 times higher than the mean molar concentrations of LSD (molecular weight: 323.4; 3.0, 3.5, 2.2 pM/mL) in the 0 to 8, 8 to 16, and 16 to 24 hour sampling intervals, respectively. Of the nonmetabolized LSD that was recovered from urine, 56% appeared in urine within the first 8 hours after administration and 45% of the O-H-LSD appeared in urine 8 to 16 hours after LSD administration. Of the orally administered LSD hydrate (200 μ g or 618 nM), 13% was eliminated in urine as O-H-LSD (28.3 μ g or 79.5 nM) within 24 hours. Only 1% (2.1 μ g or 6.4 nM) of the dose of LSD was eliminated in urine as LSD within 24 hours. The renal clearance of LSD was 1.32 ± 0.6 mL/min or approximately 1.6% of the apparent total clearance after oral administration (CL/F), assuming an oral bioavailability of 71% (see Discussion). No significant differences in LSD or O-H-LSD urine concentrations were observed between male and female subjects (Table 2). The urine recovery of O-H-LSD was greater in male subjects than in female subjects during the 8 to 16 hour sampling period, but no significant differences were observed in the overall 0 to 24 hour sampling (Table 2).

PK-Pharmacodynamic Relationship

Figure 2 shows the effects of LSD as a function of plasma concentration. There was a close relationship between the LSD concentration and its dynamic effects over time. No hysteresis was

found for heart rate (Figure 2a), blood pressure (Figure 2b), or bad drug effect (Figure 2g). The 95% CIs of the mean of the area within the hysteresis loops (A_H) overlapped with 0 for heart rate (4.4 beats \times ng/min \times mL $[-13$ to $+22]$), blood pressure (-5 mgHg \times ng/min \times mL $[-24$ to $+13]$), and bad drug effect (5% \times ng/min \times mL $[-29$ to $+38]$), indicating no hysteresis. Counterclockwise hysteresis (negative A_H value) was observed, attributable to relatively higher plasma levels compared with the dynamic effects during the assumed drug absorption phase (0–2 hours) for body temperature (Figure 2c), pupil size (Figure 2d), any drug effect (Figure 2e), and good drug effect (Figure 2f). Mean A_H values (95% CI) were the following: body temperature ($-1^\circ\text{C} \times$ ng/min \times mL $[-1.5$ to $-0.5]$), pupil size (-1.4 mm \times ng/min \times mL $[-2.2$ to $-0.7]$), any drug effect (-78% \times ng/min \times mL $[-113$ to $-43]$), and good drug effect (-106% \times ng/min \times mL $[-151$ to $-61]$). The decline of the response to LSD and plasma concentration over time followed a sigmoidal E_{max} dose-response curve for any drug effect and good drug effect. The EC_{50} mean \pm SD values were 1.3 ± 0.7 ng/mL for any drug effect and 1.0 ± 0.5 ng/mL for good drug effect. Heart rate, blood pressure, body temperature, and bad drug effect linearly increased with plasma concentrations of LSD and did not show an E_{max} (Figure 2a–c, g). Not enough values were available to fit changes in pupil size. No clockwise hysteresis was observed for any of the concentration-effect curves, meaning that the dynamic values were higher later in time at a given

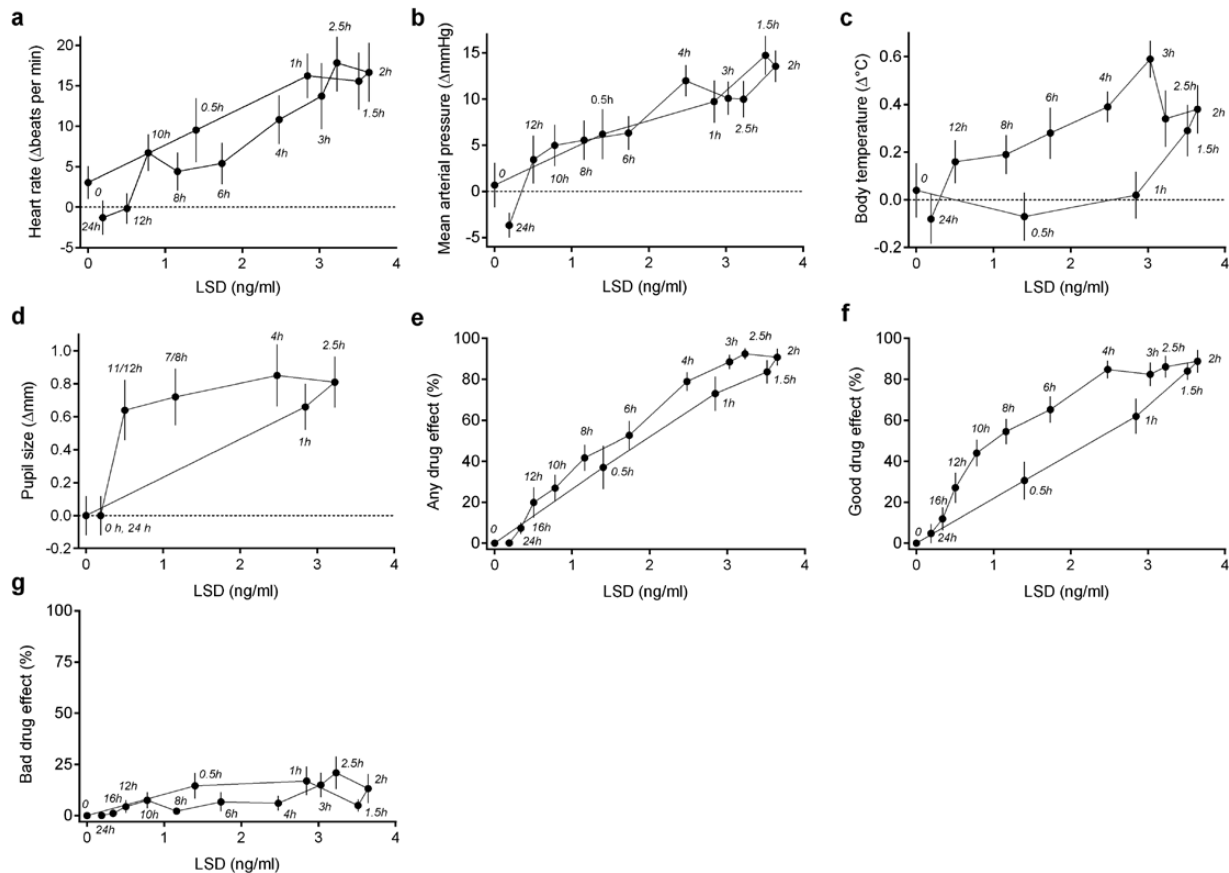


Figure 2. Lysergic acid diethylamide (LSD) effects plotted against LSD plasma concentrations (geometric means). The pharmacodynamic values are the mean \pm SEM differences from placebo at each time point in 16 subjects. The time of sampling is noted next to each point (in hours after LSD administration). Heart rate (a), mean arterial pressure (b), and bad drug effect (g) showed no hysteresis. Counterclockwise hysteresis was observed for body temperature (c), pupil size (d), any drug effect (e), and good drug effect (f), consistent with a delay between plasma concentration and effect. For most dynamic variables, maximal plasma concentrations (at approximately 2 hours) coincided with maximal dynamic effects. The dynamic changes then gradually decreased over time with decreasing plasma levels. No evidence of acute tolerance (clockwise hysteresis) was observed for any of the dynamic effects of LSD.

plasma concentration and consistent with no acute tolerance to the effects of LSD. LSD produced acute adverse effects, including difficulty concentrating, headache, exhaustion, and dizziness lasting up to 24 hours and as reported elsewhere (Schmid et al., 2014). There were no severe adverse effects.

Discussion

The present study determined the single-dose PK of oral LSD in humans. The concentrations of LSD were maximal after 1.5 hours (median) and gradually declined to very low levels by 12 hours. We observed first-order kinetics of LSD up to 12 hours in all subjects and an inconsistent slower decrease in concentrations thereafter in some subjects. This could be attributable to redistribution from tissue or due to less precise quantification of the very low plasma levels of LSD at 12 to 24 hours (ie, close to the lower limit of quantification). The half-life of 3.6 hours during the first 12 hours after drug administration is close to the 3 hours previously observed in a small study that used intravenous LSD administration (Aghajanian and Bing, 1964). Only 1% of the orally administered LSD was eliminated renally. LSD is almost completely metabolized in rats, guinea pigs, and monkeys (Axelrod et al., 1957; Siddik et al., 1979). In humans, the major metabolite of LSD detectable in urine is O-H-LSD (Klette et al., 2000; Poch et al., 2000; Canezin et al., 2001). In the present study, O-H-LSD was detected in blood plasma at very low concentrations and in only one-half of the subjects. The urine concentrations of O-H-LSD in the present study were approximately 10, 15, and 20 times higher than those of LSD at 0 to 8, 8 to 16, and 16 to 24 hours after LSD administration. Similarly, in LSD-positive forensic urine samples, O-H-LSD concentrations are higher than those of LSD, and O-H-LSD can be detected for a longer time than LSD after LSD administration (Reuschel et al., 1999; Klette et al., 2000; Poch et al., 2000). In the present study, 13% of the orally administered LSD was recovered from urine as O-H-LSD within 24 hours. LSD is metabolized to O-H-LSD by cytochrome P450 enzymes, but the specific enzymes and mechanisms are unknown (Klette et al., 2000). To our knowledge, it is unknown whether O-H-LSD is pharmacologically active.

The oral bioavailability of LSD can be crudely estimated using the previous data on intravenous LSD administration (Aghajanian and Bing, 1964) and our data on oral LSD. After intravenous LSD administration (2 µg/kg of the free base in 5 male subjects), a mean total plasma exposure (AUC_{∞}) of 31.4 ng·mL/h was obtained (15.7 ng·mL/h per µg/kg free base), calculated based on the published plasma concentration profile (Aghajanian and Bing, 1964). After oral LSD administration in the present study (2.5 µg/kg free base in 8 male subjects), the mean AUC_{∞} was 28 ng·mL/h (11.2 ng·mL/h per µg/kg free base). Based on these data, the oral bioavailability of LSD is approximately 71%. In the present study, LSD was administered after a light meal. When ingested with a "full breakfast," oral LSD was reported to result in lower plasma concentrations compared with administration on an empty stomach (Upshall and Wailling, 1972). However, these observations were made in only 2 to 3 subjects (Upshall and Wailling, 1972) and would need confirmation. Remaining to be tested is whether food reduces or delays the absorption of oral LSD. Additionally, the PK profiles were similar in male and female subjects. However, the study was too underpowered to statistically exclude sex differences in the PK of LSD.

We found a close relationship between the plasma concentrations of LSD and physiologic response or psychotropic effects of LSD over time. Estimated EC_{50} values for the psychotropic effects were in the range of 1.0 to 1.3 ng/mL (approximately 3–4 nM). The unbound fraction of LSD in human plasma

is unknown. In cats, the unbound fraction was 0.2, and LSD concentrations in cerebrospinal fluid were similar to free LSD plasma concentrations (Axelrod et al., 1957). Thus, LSD concentrations of 0.6 to 0.8 nM could be expected in cerebrospinal fluid. These values are in the range of the binding affinity of LSD at the 5-hydroxytryptamine-2A (5-HT_{2A}) receptor (K_i = 0.4–1.3 nM, respectively) (Titeler et al., 1988; Egan et al., 1998) and also close to the EC_{50} for the functional stimulant activity of LSD at the receptor in vitro (EC_{50} = 7.2 nM) (Egan et al., 1998). Pupil size was also strongly increased at low concentrations of LSD. We previously showed that pupil diameters were significantly larger compared with placebo until the last pupil measurement at 11 hours after LSD administration. In contrast, elevations in blood pressure, heart rate, and body temperature were only significant up to 5 hours after LSD administration compared with placebo, as reported elsewhere (Schmid et al., 2014). Additionally, the increases in heart rate, blood pressure, body temperature, and bad drug effects showed no ceiling effect in the concentration-effect curves, in contrast to the other dynamic effects of LSD. Heart rate, body temperature, blood pressure, and bad drug effects would likely increase further with higher doses of LSD, whereas the pupillary or good subjective effects can be expected to be similar to those seen in the present study. The hypertensive effects of LSD may result from 5-HT_{2A} and/or α_1 -adrenergic receptor-mediated vasoconstrictive effects at higher doses (Dyer and Gant, 1973; Blessing and Seaman, 2003).

No evidence of acute tolerance was observed, which would become apparent as clockwise hysteresis in the concentration-response curve and has been shown for 3,4-methylenedioxymethamphetamine (MDMA) (Hysek et al., 2011). In contrast and as typically expected for most drugs, counterclockwise hysteresis was observed early in time until the end of the assumed drug absorption phase. No similar studies on the PK-pharmacodynamic relationship of LSD have been performed. Only one other small study measured plasma LSD concentrations and concomitant pharmacodynamic effects (Aghajanian and Bing, 1964). LSD was administered intravenously in 5 male subjects. To obtain a crude index of performance, subjects were given one of a series of equivalent tests, consisting of simple addition problems, after each blood sample was drawn (Aghajanian and Bing, 1964). After the distribution phase (30 minutes after intravenous LSD administration), the impairments in performance declined in parallel with the plasma levels of LSD, also suggesting a close temporal relationship between the PK and pharmacodynamics of LSD (Aghajanian and Bing, 1964). In contrast to the single-dose administration in the present study, tolerance to the subjective effects of LSD with repeated daily LSD administration has been reported (Abramson et al., 1956; Belleville et al., 1956). However, a gradual increase in head twitches and catatonic postures and no tolerance was observed up to 3 to 4 days after continuous LSD administration in rats (Ellison et al., 1980). Also in contrast to our findings with LSD, we observed pronounced acute tolerance to the psychotropic and cardiostimulant effects of MDMA using the same methodology (Hysek et al., 2011). As a result, the pharmacodynamic effects of MDMA last significantly shorter than would be expected based on plasma levels. The subjective and cardiostimulant effects of MDMA last only 5 hours despite its long half-life of 10 hours (Hysek et al., 2011). In contrast, the subjective drug effects of LSD lasted for 12 hours in most subjects and up to 16 hours in some subjects in the present study despite LSD's shorter half-life. Thus, subjects with MDMA in blood may no longer be clinically intoxicated, whereas subjects with quantifiable LSD concentrations in plasma are clinically intoxicated.

A mechanistic explanation for this acute tolerance in the case of MDMA is that it mainly produces its acute effects through the release of endogenous serotonin and norepinephrine (ie, as an indirect serotonergic and noradrenergic agonist). In contrast, LSD is thought to produce its psychotropic hallucinogenic effects through a direct interaction with the 5-HT_{2A} receptor (ie, as a direct serotonergic agonist), resulting in pharmacodynamic effects to which no acute tolerance was observed in our study.

In summary, we show first data on the PK and PK-pharmacodynamic relationship of oral LSD in human subjects. The PK profiles exhibit first-order kinetics of LSD up to 12 hours. LSD produces physiological and psychotropic effects lasting up to 12 hours, closely related to the plasma concentrations of LSD and inhibiting no acute tolerance. The findings are important for further clinical studies and serve as a reference for the assessment of intoxication with LSD.

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Statement of Interest

The authors declare no competing financial interests.

References

- Abramson HA, Jarvik ME, Gorin MH, Hirsch MW (1956) Lysergic acid diethylamide (LSD-25): XVII tolerance development and its relationship to a theory of psychosis. *J Psychol* 41:81–105.
- Aghajanian GK, Bing OH (1964) Persistence of lysergic acid diethylamide in the plasma of human subjects. *Clin Pharmacol Ther* 5:611–614.
- Axelrod J, Brady RO, Witkop B, Evarts EV (1957) The distribution and metabolism of lysergic acid diethylamide. *Ann N Y Acad Sci* 66:435–444.
- Belleville RE, Fraser HF, Isbell H, Wikler A, Logan CR (1956) Studies on lysergic acid diethylamide (LSD-25): I. Effects in former morphine addicts and development of tolerance during chronic intoxication. *AMA Arch Neurol Psychiatry* 76:468–478.
- Blessing WW, Seaman B (2003) 5-hydroxytryptamine_{2A} receptors regulate sympathetic nerves constricting the cutaneous vascular bed in rabbits and rats. *Neuroscience* 117:939–948.
- Canezin J, Cailleux A, Turcant A, Le Bouil A, Harry P, Allain P (2001) Determination of LSD and its metabolites in human biological fluids by high-performance liquid chromatography with electrospray tandem mass spectrometry. *J Chromatogr B Biomed Sci Appl* 765:15–27.
- Dolder PC, Liechti ME, Rentsch KM (2015) Development and validation of a rapid turboflow LC-MS/MS method for the quantification of LSD and 2-oxo-3-hydroxy LSD in serum and urine samples of emergency toxicological cases. *Anal Bioanal Chem* 407:1577–84.
- Dyer DC, Gant DW (1973) Vasoconstriction produced by hallucinogens on isolated human and sheep umbilical vasculature. *J Pharmacol Exp Ther* 184:366–375.
- Egan CT, Herrick-Davis K, Miller K, Glennon RA, Teitler M (1998) Agonist activity of LSD and lisuride at cloned 5HT_{2A} and 5HT_{2C} receptors. *Psychopharmacology* 136:409–414.
- Ellison G, Ring M, Ross D, Axelrod B (1980) Cumulative alterations in rat behavior during continuous administration of LSD or mescaline: absence of tolerance? *Biol Psychiatry* 15:95–102.
- Gasser P, Holstein D, Michel Y, Doblin R, Yazar-Klosinski B, Passie T, Brenneisen R (2014) Safety and efficacy of lysergic acid diethylamide-assisted psychotherapy for anxiety associated with life-threatening diseases. *J Nerv Ment Dis* 202:513–520.
- Hysek CM, Liechti ME (2012) Effects of MDMA alone and after pretreatment with reboxetine, duloxetine, clonidine, carvedilol, and doxazosin on pupillary light reflex. *Psychopharmacology* 224:363–376.
- Hysek CM, Simmler LD, Ineichen M, Grouzmann E, Hoener MC, Brenneisen R, Huwyler J, Liechti ME (2011) The norepinephrine transporter inhibitor reboxetine reduces stimulant effects of MDMA (“ecstasy”) in humans. *Clin Pharmacol Ther* 90:246–255.
- Hysek CM, Simmler LD, Schillinger N, Meyer N, Schmid Y, Donzelli M, Grouzmann E, Liechti ME (2014) Pharmacokinetic and pharmacodynamic effects of methylphenidate and MDMA administered alone and in combination. *Int J Neuropsychopharmacol* 17:371–381.
- Klette KL, Anderson CJ, Poch GK, Nimrod AC, ElSohly MA (2000) Metabolism of lysergic acid diethylamide (LSD) to 2-oxo-3-hydroxy LSD (O-H-LSD) in human liver microsomes and cryopreserved human hepatocytes. *J Anal Toxicol* 24:550–556.
- Klette KL, Horn CK, Stout PR, Anderson CJ (2002) LC-MS analysis of human urine specimens for 2-oxo-3-hydroxy LSD: method validation for potential interferants and stability study of 2-oxo-3-hydroxy LSD under various storage conditions. *J Anal Toxicol* 26:193–200.
- Kupferschmidt K (2014) High hopes. *Science* 345:18–23.
- Martin R, Schurenkamp J, Gasse A, Pfeiffer H, Kohler H (2013) Determination of psilocin, bufotenine, LSD and its metabolites in serum, plasma and urine by SPE-LC-MS/MS. *Int J Legal Med* 127:593–601.
- Nichols DE (2004) Hallucinogens. *Pharmacol Ther* 101:131–181.
- Passie T, Halpern JH, Stichtenoth DO, Emrich HM, Hintzen A (2008) The pharmacology of lysergic acid diethylamide: a review. *CNS Neurosci Ther* 14:295–314.
- Poch GK, Klette KL, Anderson C (2000) The quantitation of 2-oxo-3-hydroxy lysergic acid diethylamide (O-H-LSD) in human urine specimens, a metabolite of LSD: comparative analysis using liquid chromatography-selected ion monitoring mass spectrometry and liquid chromatography-ion trap mass spectrometry. *J Anal Toxicol* 24:170–179.
- Reuschel SA, Eades D, Foltz RL (1999) Recent advances in chromatographic and mass spectrometric methods for determination of LSD and its metabolites in physiological specimens. *J Chromatogr B Biomed Sci Appl* 733:145–159.
- Schmid Y, Enzler F, Gasser P, Grouzmann E, Preller KH, Vollenweider FX, Brenneisen R, Müller F, Borgwardt S, Liechti ME (2014) Acute effects of lysergic acid diethylamide in healthy subjects. *Biol Psychiatry* doi: 10.1016/j.biopsych.2014.11.015.
- Siddik ZH, Barnes RD, Dring LG, Smith RL, Williams RT (1979) The fate of lysergic acid DI[14C]ethylamide ([14C]LSD) in the rat, guinea pig and rhesus monkey and of [14C]iso-LSD in rat. *Biochem Pharmacol* 28:3093–3101.
- Titeler M, Lyon RA, Glennon RA (1988) Radioligand binding evidence implicates the brain 5-HT₂ receptor as a site of action for LSD and phenylisopropylamine hallucinogens. *Psychopharmacology* 94:213–216.
- Upshall DG, Wailling DG (1972) The determination of LSD in human plasma following oral administration. *Clin Chim Acta* 36:67–73.

3.3 Publication 3

Alterations of consciousness and mystical-type experiences after acute LSD in humans

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Alterations of consciousness and mystical-type experiences after acute LSD in humans

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Abstract

Rationale Lysergic acid diethylamide (LSD) is used recreationally and in clinical research. Acute mystical-type experiences that are acutely induced by hallucinogens are thought to contribute to their potential therapeutic effects. However, no data have been reported on LSD-induced mystical experiences and their relationship to alterations of consciousness. Additionally, LSD dose- and concentration-response functions with regard to alterations of consciousness are lacking.

Methods We conducted two placebo-controlled, double-blind, cross-over studies using oral administration of 100 and 200 µg LSD in 24 and 16 subjects, respectively. Acute effects of LSD were assessed using the 5 Dimensions of Altered States of Consciousness (5D-ASC) scale after both doses and the Mystical Experience Questionnaire (MEQ) after 200 µg.

Results On the MEQ, 200 µg LSD induced mystical experiences that were comparable to those in patients who underwent LSD-assisted psychotherapy but were fewer than those reported for psilocybin in healthy subjects or patients.

On the 5D-ASC scale, LSD produced higher ratings of blissful state, insightfulness, and changed meaning of percepts after 200 µg compared with 100 µg. Plasma levels of LSD were not positively correlated with its effects, with the exception of ego dissolution at 100 µg.

Conclusions Mystical-type experiences were infrequent after LSD, possibly because of the set and setting used in the present study. LSD may produce greater or different alterations of consciousness at 200 µg (i.e., a dose that is currently used in psychotherapy in Switzerland) compared with 100 µg (i.e., a dose used in imaging studies). Ego dissolution may reflect plasma levels of LSD, whereas more robustly induced effects of LSD may not result in such associations.

Keywords LSD · Altered states of consciousness · Mystical experiences

The studies were registered at ClinicalTrials.gov (NCT02308969, NCT01878942).

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Introduction

Lysergic acid diethylamide (LSD) is the prototypical hallucinogen (Nichols 2016; Passie et al. 2008). LSD became famous, with a high cultural influence, in the 1960s. LSD continues to be used for recreational and personal purposes (Krebs and Johansen 2013). Additionally, there is much interest in its therapeutic potential (Baumeister et al. 2014; Davenport 2016; Gasser et al. 2014; Gasser et al. 2015; Krebs and Johansen 2012; Kupferschmidt 2014). Only one modern study has tested the therapeutic effects of LSD in patients (Gasser et al. 2014), whereas several clinical trials have recently evaluated the therapeutic potential of psilocybin (Bogenschutz et al. 2015; Carhart-Harris et al. 2016a; Garcia-Romeu et al. 2015; Griffiths 2016; Grob et al. 2011; Guss 2016), a similar serotonergic hallucinogen (Rickli et al. 2016). A series of studies showed that psilocybin acutely

induced mystical experiences in healthy subjects and patients (Garcia-Romeu et al. 2015; Griffiths et al. 2008; Griffiths et al. 2011; Griffiths et al. 2006; MacLean et al. 2011). Additionally, greater acute effects of psilocybin on the Mystical Experience Questionnaire (MEQ; Barrett et al. 2015; Griffiths et al. 2006; MacLean et al. 2012) were associated with positive long-term effects on mood and personality in healthy subjects (Griffiths et al. 2008; Griffiths et al. 2011; Griffiths et al. 2006; MacLean et al. 2011) and better therapeutic outcomes in patients with anxiety, depression, and substance use disorder (Garcia-Romeu et al. 2015; Griffiths 2016; Griffiths et al. 2008; Griffiths et al. 2011; Griffiths et al. 2006; MacLean et al. 2011). Early studies reported on mystical experiences after experimental administration of LSD, but methodological details are missing (Turek et al. 1974). Whether and the extent to which LSD produces mystical-type effects in the MEQ are currently unknown. Therefore, we characterized the effects of 200 µg LSD on the MEQ and evaluated the way in which mystical experiences are related to LSD-induced increases in 5 Dimensions of Altered States of Consciousness (5D-ASC) scale scores and plasma levels of LSD.

Clinical experimental research with LSD has recently seen a resurgence (Carhart-Harris et al. 2016b; Carhart-Harris et al. 2015; Carhart-Harris et al. 2016c; Dolder et al. 2015b; Dolder et al. 2016; Kaelen et al. 2015; Kaelen et al. 2016; Lebedev et al. 2016; Roseman et al. 2016; Schmid et al. 2015; Speth et al. 2016; Strajhar et al. 2016; Tagliazucchi et al. 2016; Terhune et al. 2016). An increasing amount of data has been generated on the effects of LSD (75 µg) on various neuronal correlates of brain activation (Carhart-Harris et al. 2016c; Kaelen et al. 2016; Lebedev et al. 2016; Roseman et al. 2016). Researchers have correlated subjective drug effects with brain functional magnetic resonance imaging (fMRI) data (Carhart-Harris et al. 2016c; Kaelen et al. 2016; Lebedev et al. 2016; Roseman et al. 2016). This approach likely produces significant findings for subjective effects that show large between-subject variance but not for subjective effects of the substance that are consistently present in all subjects. Lower doses of LSD may also result in more variable responses across subjects compared with higher doses. Furthermore, higher doses of LSD (e.g., 200 µg) that are currently used therapeutically (Gasser et al. 2014) may produce more pronounced but also qualitatively different subjective effects (Dolder et al. 2016). Importantly, plasma concentrations of LSD have not been determined in any of the published LSD fMRI studies to date; therefore, unclear is the way in which LSD exposure in the body is linked to subjective effects in these studies. Therefore, a second goal of the present study was to describe the subjective peak effects of two doses of LSD (100 and 200 µg) using the 5D-ASC scale (Studerus et al. 2010). The 5D-ASC scale has been used in all of the recent experimental studies with LSD (Carhart-Harris et al. 2016b; Carhart-Harris et al. 2016c; Schmid et al. 2015; Tagliazucchi et al. 2016) and

with many other psychedelics, providing an opportunity to compare findings between studies and across substances and research groups. Thus, the present study assessed LSD dose- and plasma concentration-response functions using the 5D-ASC scale in 40 subjects (Dolder et al. 2015b; Dolder et al. 2016; Schmid et al. 2015), thus allowing comparisons with other studies that used the 5D-ASC scale but did not determine plasma LSD concentrations (Carhart-Harris et al. 2016b; Carhart-Harris et al. 2016c; Kaelen et al. 2016; Lebedev et al. 2016; Roseman et al. 2016; Speth et al. 2016; Tagliazucchi et al. 2016; Terhune et al. 2016). A third goal of the present study was to assess associations across subjects between the peak and total plasma exposure to LSD and its effects on 5D-ASC scale scores (Studerus et al. 2010). The effects of 100 µg LSD on 5D-ASC scale scores are reported for the first time in the present study, whereas the effects of 200 µg have been previously published (Schmid et al. 2015). However, the latter study did not evaluate dose- or concentration-response functions. Other data that were generated in the present study have been previously reported including acute and subacute adverse effects (Dolder et al. 2015b; Dolder et al. 2016; Schmid et al. 2015; Strajhar et al. 2016).

Material and methods

Study design

We performed two similar studies using double-blind, placebo-controlled, cross-over designs with two experimental test sessions (LSD and placebo) in a balanced order. Study 1 used a dose of 100 µg LSD and placebo in 24 subjects. Study 2 used 200 µg LSD and placebo in 16 subjects. The washout periods between sessions were at least 7 days. The studies were conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee. The administration of LSD to healthy subjects was authorized by the Swiss Federal Office for Public Health, Bern, Switzerland. All of the subjects provided written consent before participating in either of the studies, and they were paid for their participation. The studies were registered at [ClinicalTrials.gov](https://clinicaltrials.gov) (NCT02308969, NCT01878942).

Participants

Forty healthy participants were recruited from the University of Basel campus via online advertisement. Twenty-four subjects (12 men, 12 women; 33 ± 11 years old [mean \pm SD]; range, 25–60 years) participated in study 1, and 16 subjects (8 men, 8 women; 29 ± 6 years old; range, 25–51 years) participated in study 2. The inclusion and exclusion criteria were identical for both studies. Subjects younger than 25 years of age were excluded from participating in the study. Additional exclusion criteria were age >65 years, pregnancy (urine

pregnancy test at screening and before each test session), personal or family (first-degree relative) history of major psychiatric disorders (assessed by the semi-structured clinical interview for *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition, Axis I disorders by the study physician and an additional interview by a trained psychiatrist), use of medications that may interfere with the study medication, chronic or acute physical illness (abnormal physical exam, electrocardiogram, or hematological and chemical blood analyses), tobacco smoking (>10 cigarettes/day), lifetime prevalence of illicit drug use >10 times (except for tetrahydrocannabinol), illicit drug use within the last 2 months, and illicit drug use during the study (determined by urine drug tests). The subjects were asked to abstain from excessive alcohol consumption between test sessions and particularly limit their use to one standard drink on the day before the test sessions. Additionally, the participants were not allowed to drink xanthine-containing liquids after midnight before the study day. Eleven subjects had used a hallucinogen, including LSD (six participants), one to three times, and most of the subjects (29) were hallucinogen-naïve. We performed urine drug tests at screening and before each test session, and no substances were detected during the study.

Study procedures

Each study included a screening visit, a psychiatric interview, two 25-h experimental sessions, and an end-of-study visit. The experimental sessions were conducted in a quiet standard hospital patient room. The participants were resting in hospital beds except when going to the restroom. Only one research subject and one or two investigators were present during the experimental sessions. The participants could interact with the investigator, rest quietly, and/or listen to music via headphones, but no other entertainment was provided. LSD or placebo was administered at 9:00 AM. The subjects were never alone during the first 12 h after drug administration, and the investigator was in a room next to the subject for up to 24 h while the subjects were asleep (mostly from 1:00 AM to 8:00 AM).

Study drug

LSD (*d*-LSD hydrate, HPLC purity >99 %, Lipomed AG, Arlesheim, Switzerland) was administered in single oral doses of 100 or 200 µg as gelatin capsules. Note that these LSD hydrate doses correspond to LSD tartrate doses of 123 and 246 µg, respectively. In the 1960–1970s, small doses of LSD tartrate of 25–150 µg were typically used in “psycholytic therapy” and higher doses of >200 µg in “psychedelic” therapy (Pahnke et al. 1970). The dose used in a recent LSD-assisted psychotherapy study was 200 µg LSD hydrate (Gasser et al. 2014). Both doses used in the present study were within the range of doses that are taken for recreational

purposes (Passie et al. 2008). Corresponding placebo capsules were used.

Measures

Mystical-type experiences In study 2, mystical experiences were assessed using a German version (Supplementary Appendix 1) of the 43-item MEQ (Griffiths et al. 2006; MacLean et al. 2012; Pahnke 1969) embedded in the 100-item States of Consciousness Questionnaire (SOCQ; (Griffiths et al. 2006). The original English questionnaire was independently forward-translated into German by two translators with German as their mother tongue. Discrepancies between the two forward-translated versions and a previous German version were then discussed and selected items backtranslated. The version was then pretested for comprehension by persons with previous LSD or MDMA use.

The MEQ has been used in numerous experimental and therapeutic trials with psilocybin (Garcia-Romeu et al. 2015; Griffiths et al. 2008; Griffiths et al. 2011; Griffiths et al. 2006; MacLean et al. 2011). The MEQ items provide scale scores for each of seven domains of mystical experiences: internal unity, external unity, sacredness, noetic quality (as real as or more real than everyday reality), deeply felt positive mood, transcendence of time and space, and ineffability/paradoxicality (difficulty describing the experience in words). The total of all scale scores was used as an overall measure of the mystical-type experience. We also derived the four scale scores of the newly validated revised 30-item MEQ: mystical, positive mood, transcendence of time and space, and ineffability (Barrett et al. 2015). A complete mystical experience was defined as scores ≥ 60 % on all MEQ30 factors (Barrett et al. 2015). The MEQ was administered 24 h after drug administration, and the participants were asked to retrospectively rate drug effects during peak drug effects. For comparison, we included MEQ ratings that were obtained 6 h after administration of 3,4-methylenedioxymethamphetamine (MDMA) and methylphenidate in another study using a similar research setting (Schmid et al. 2014). Additionally, we included MEQ ratings from patients who were treated with 200 µg LSD for anxiety related to life-threatening illness in another study (Diesch 2015; Gasser et al. 2014; Gasser et al. 2015). All of these additional MEQ findings have not been previously published in scientific journals and were obtained in studies that were previously described in detail (Diesch 2015; Gasser et al. 2014; Gasser et al. 2015; Schmid et al. 2014).

Alterations of consciousness The 5D-ASC scale was used in both studies to assess the overall peak alterations of consciousness. The 5D-ASC scale measures altered states of consciousness and contains 94 items (visual analog scales). The instrument consists of five subscales/dimensions (Dittrich 1998) and 11 lower-order scales (Studerus et al. 2010). The 5D-ASC dimension “Oceanic Boundlessness” (27 items)

measures derealization and depersonalization associated with positive emotional states, ranging from heightened mood to euphoric exaltation. The corresponding lower-order scales include “experience of unity,” “spiritual experience,” “blissful state,” and “insightfulness.” The dimension “Anxious Ego Dissolution” (21 items) summarizes ego disintegration and loss of self-control phenomena associated with anxiety. The corresponding lower-order scales include “disembodiment,” “impaired control of cognition,” and “anxiety.” The dimension “Visionary Restructuralization” (18 items) consists of the lower-order scales “complex imagery,” “elementary imagery,” “audio-visual synesthesia,” and “changed meaning of percepts.” Two additional dimensions describe “Auditory Alterations” (15 items) and “Reduction of Vigilance” (12 items). The scale is well-validated and widely used to characterize the subjective effects of various psychedelic drugs (Carhart-Harris et al. 2016b; Hasler et al. 2004; Hysek et al. 2011; Schmid et al. 2015; Vollenweider et al. 2007; Vollenweider and Kometer 2010). In addition to the subscale analyses, we also analyzed the effects on ego dissolution item 71 (the boundaries between myself and my surroundings seemed to blur) because the concept of ego dissolution was often used in recent imaging studies (Tagliazucchi et al. 2016). The 5D-ASC scale was administered 24 h after drug administration, and the participants were asked to retrospectively rate the drug effects. 5D-ASC ratings were also performed at 3 and 10 h in study 1.

Analysis of plasma LSD concentrations

Blood was collected into lithium heparin tubes before and 0.5, 1, 1.5, 2.5, 3, 4, 6, 8, 10, 12, 16, and 24 h after LSD administration. The 0.5, 1.5, and 2.5 h samples were not collected in study 1. Blood samples were immediately centrifuged, and the plasma was rapidly stored at -20°C and later analyzed using liquid-chromatography-tandem mass-spectrometry as previously reported (Dolder et al. 2015a; Steuer et al. 2016). Maximal plasma concentrations (C_{max}) and total exposure (area under the plasma concentration-time curve [AUC]) were estimated using compartmental modeling in Phoenix WinNonlin 6.4 (Certara, Princeton, NJ, USA). A one-compartment model was used with first-order input, first-order elimination, and no lag time.

Statistical analyses

The data analysis was performed using Statistica 12 software (StatSoft, Tulsa, OK, USA). Differences between LSD and placebo or between the 100 and 200 μg doses of LSD were compared using dependent or independent t tests, respectively. Associations between outcome measures were assessed using Pearson correlations. Significance was assumed at $p < 0.05$.

Results

Mystical-type experiences

LSD (200 μg) significantly increased all MEQ scores compared with placebo (Fig. 1a, Table 1). The effects of MDMA and methylphenidate on MEQ scores are included for comparison (Fig. 1a). The effects of LSD (200 μg) and placebo on MEQ scores in 11 patients during LSD-assisted psychotherapy (Gasser et al. 2014) are also shown in Fig. 1b. LSD-induced mystical experiences were comparable in healthy subjects in the laboratory setting in the present study and in patients in the therapeutic setting (Fig. 1b). Only two subjects in each of the studies had a complete mystical experience. The MEQ30 total scores were $<5\%$ in both settings after placebo administration (Fig. 1b).

Alterations of consciousness

LSD induced pronounced peak alterations of waking consciousness, with significant increases in all dimensions and subscales of the 5D-ASC scale (Fig. 2). The 200 μg dose of LSD produced significantly greater scores on the overall ASC scale, the dimension of visionary restructuring, and the blissful state, insightfulness, and changed meaning of percepts subscales compared with the 100 μg dose (Fig. 2, Table 1). The mean \pm SEM ego dissolution (item 71) scores were 49 ± 6 and 53 ± 10 after the 100 and 200 μg doses, respectively (Table 1). There were only minimal differences between the 5D-ASC ratings at 3, 10, and 24 h (supplementary Fig. S1 online).

Plasma LSD concentrations

Plasma concentrations varied between subjects, especially at the lower 100 μg dose. The median (range) C_{max} values were 1.4 ng/ml (0.32–3.7) and 3.2 ng/ml (1.9–7.1) for the 100 and 200 μg doses, respectively. The corresponding AUC values were $8.5 \text{ ng} \times \text{h/ml}$ (1–19) and $20.7 \text{ ng} \times \text{h/ml}$ (11–39).

Associations between alterations of consciousness and mystical-type experiences

Table 2 shows the cross-tabulation of all correlations between the 5D-ASC scale and MEQ30 subscale ratings. LSD-induced alterations of consciousness (ASC total score) were significantly correlated with ratings of mystical experience (MEQ30 total score) on the MEQ ($R_p = 0.87$, $p < 0.001$, $n = 16$; Fig. 3). Scores on the MEQ positive mood scale were strongly associated with scores on the ASC experience of unity and blissful state scales ($R_p = 0.85$ and 0.80 , respectively; both $p < 0.001$, $n = 16$; Table 2).

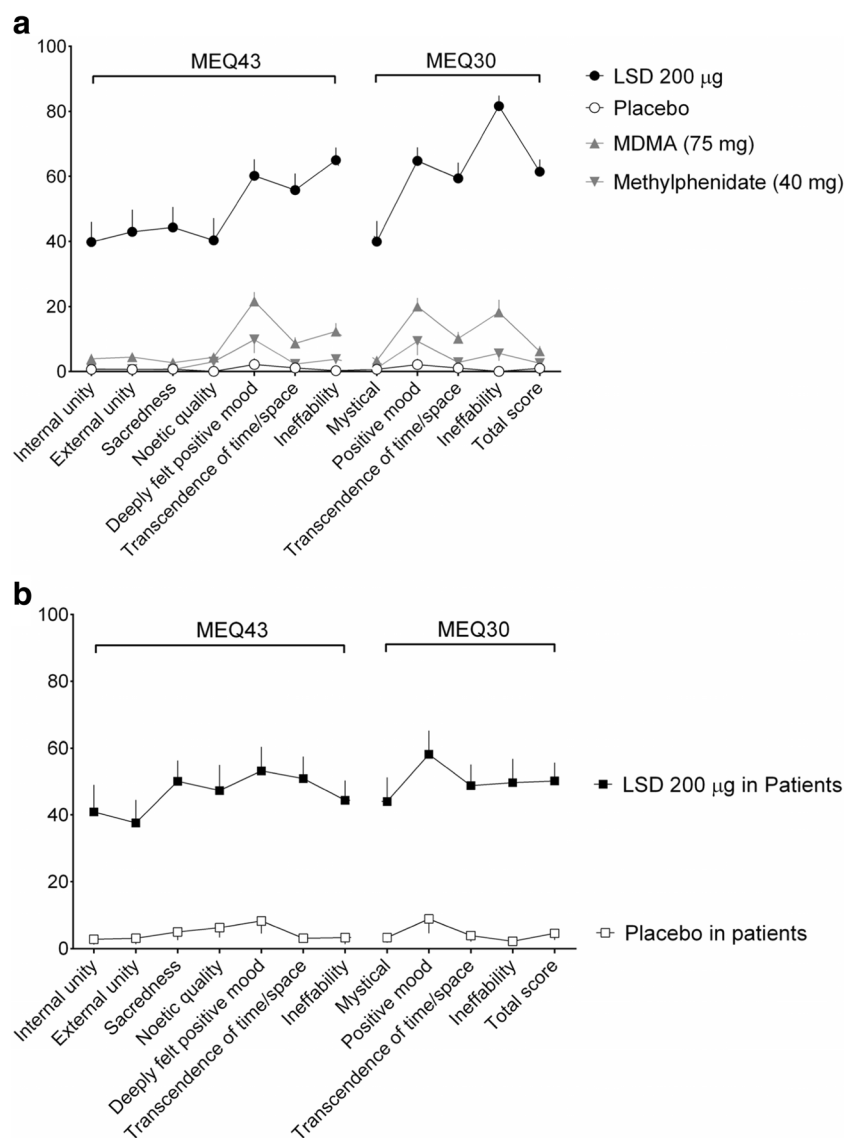


Fig. 1 Effects of LSD on the Mystical Experience Questionnaire (MEQ). **a** In the present study in healthy subjects, LSD (200 µg) significantly increased scores on all scales of the MEQ43 and MEQ30 compared with placebo (Table 1). The data are expressed as the mean \pm SEM in 16 subjects. For comparison, 3,4-methylenedioxymethamphetamine (MDMA; 75 mg) and methylphenidate (40 mg) produced small increases in MEQ ratings in 30 different participants in another study in the same research setting (Schmid et al. 2014). **b** Effects of LSD on the MEQ in patients with anxiety in the context of life-threatening illness. The data were analyzed identically to the data that were obtained in the present study. The study and patient characteristics have been previously published in detail (Diesch 2015; Gasser et al. 2014; Gasser et al. 2015;

Schmid et al. 2014). Similar to the present study, the MEQ was administered on the day after LSD (200 µg) or active placebo (25 µg LSD) administration and was embedded into the larger 100-item States of Consciousness Questionnaire (SOCQ; Griffiths et al. 2006). The patient data are expressed as the mean \pm SEM in 11 subjects for LSD (200 µg, same formulation as in the present study) and four subjects for placebo. On the 43- and 30-item versions of the MEQ, LSD (200 µg) increased MEQ rating scores in the patients in the therapeutic setting (**b**) to a similar extent as in the healthy subjects in the present study (**a**). Notably, the placebo response (a very low dose of LSD of 25 µg was used as the active placebo) in the patients was small (**b**), which was also similar to the response in healthy subjects in the present study (**a**)

Correlations between plasma LSD concentrations and LSD-induced alterations of consciousness and mystical-type experiences

The C_{\max} and AUC values for LSD were not positively correlated with ratings of peak subjective effects on the 5D-ASC scale or MEQ across subjects or within dose

groups (Table 3). For example, LSD induced consistently high ratings of audio-visual synesthesia in almost all of the subjects at the high dose (200 µg), resulting in little within-subject variance and no association with plasma exposure to LSD (Table 3, Fig. 4a). One exception was ego dissolution (item 71) at the lower dose of LSD (100 µg; Table 3, Fig. 4b). The ratings showed high

Table 1 Statistics for the effects of LSD in the 5D-ASC and MEQ

	LSD 100 µg		LSD 200 µg		LSD 100 vs. 200 µg	
	<i>T</i> test vs. placebo		<i>T</i> test vs. placebo		<i>T</i> test	
	<i>T</i> =	<i>P</i> =	<i>T</i> =	<i>P</i> =	<i>T</i> =	<i>P</i> =
5 Dimensions Altered States of Consciousness (ASC) scale						
Total ASC score	9.72	<0.001	10.02	<0.001	2.23	<0.05
Oceanic boundlessness	8.44	<0.001	9.61	<0.001	1.89	NS
Anxious ego dissolution	6.43	<0.001	4.01	<0.001	1.50	NS
Visionary restructuralization	9.79	<0.001	15.32	<0.001	2.34	<0.05
Auditory alterations	3.72	<0.01	5.87	<0.001	0.42	NS
Reductions of vigilance	7.44	<0.001	5.93	<0.001	0.79	NS
Experience of unity	6.85	<0.001	7.77	<0.001	0.68	NS
Spiritual experience	4.31	<0.001	3.91	<0.001	1.10	NS
Blissful state	6.56	<0.001	8.27	<0.001	3.00	<0.01
Insightfulness	4.11	<0.001	5.81	<0.001	2.28	<0.05
Disembodiment	6.93	<0.001	5.87	<0.001	0.13	NS
Impaired control and cognition	7.01	<0.001	5.04	<0.001	0.86	NS
Anxiety	3.02	<0.001	2.04	NS	1.37	NS
Complex imagery	7.10	<0.001	7.48	<0.001	0.31	NS
Elementary imagery	9.96	<0.001	11.12	<0.001	0.57	NS
Audio-visual synesthesia	9.19	<0.001	12.52	<0.001	1.96	NS
Changed meaning of percepts	6.25	<0.001	9.66	<0.001	3.39	<0.01
Ego dissolution (item 71)	7.63	<0.001	5.32	<0.001	0.36	NS
Mystical Effects Questionnaire (MEC43)						
Internal unity	NA	NA	6.22	<0.001	NA	NA
External unity	NA	NA	6.08	<0.001	NA	NA
Sacredness	NA	NA	6.80	<0.001	NA	NA
Noetic quality	NA	NA	5.71	<0.001	NA	NA
Deeply felt positive mood	NA	NA	11.43	<0.001	NA	NA
Transcendence of time/space	NA	NA	10.63	<0.001	NA	NA
Ineffability	NA	NA	16.22	<0.001	NA	NA
Mystical Effects Questionnaire (MEQ30)						
Mystical	NA	NA	5.99	<0.001	NA	NA
Positive mood	NA	NA	13.13	<0.001	NA	NA
Transcendence of time/space	NA	NA	11.12	<0.001	NA	NA
Ineffability	NA	NA	25.14	<0.001	NA	NA
MEC30 total score	NA	NA	14.91	<0.001	NA	NA

Sixteen subjects participated in the high-dose study (200 µg) and 24 subjects in the moderate-dose study (100 µg). Dependent *T* tests were performed to assess differences from placebo, and independent *T* tests were performed to assess differences between doses of LSD

NA not assessed

interindividual variance, and there was a significant positive correlation with the LSD AUC value in the 100 µg dose group ($R_p = 0.51$, $p < 0.05$, $n = 16$; Table 3, Fig. 4b). At the 200 µg dose, there were significant negative correlations between C_{\max} values for LSD and subjective effects on the 5D-ASC scale including visionary restructuralization, elementary imagery, and changed meaning of percepts.

Discussion

The present study characterized LSD-induced mystical experiences using the MEQ after a dose of 200 µg and alterations of consciousness on the 5D-ASC scale after a dose of 100 µg. The study also evaluated associations between plasma LSD concentrations and these subjective effects.

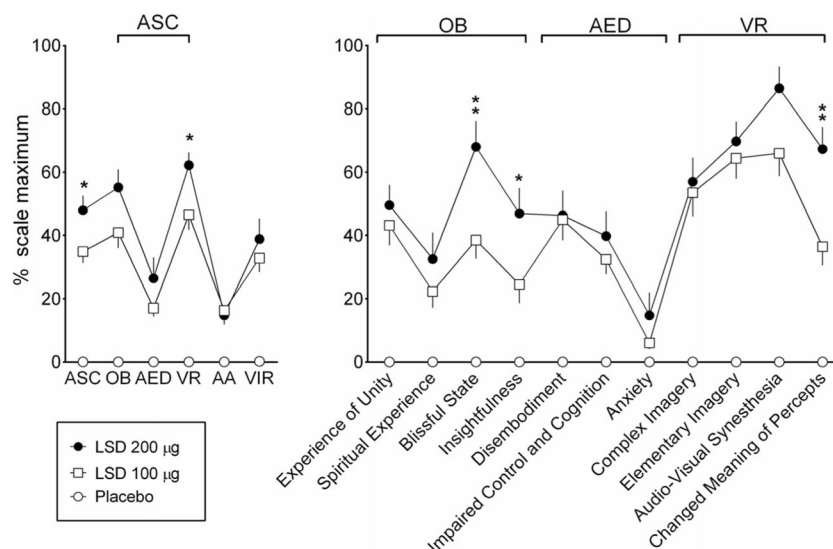


Fig. 2 Effects of LSD on the 5 Dimensions of Altered States of Consciousness (5D-ASC) scale. LSD mainly increased ratings of oceanic boundlessness (OB) and visionary restructuralization (VR), with significantly higher ratings for the ASC total score and VR dimension at 200 µg compared with 100 µg. LSD-induced increases in anxious ego dissolution (AED) and auditory alterations (AA) were relatively small. LSD also produced vigilance reduction (VIR). LSD-induced changes on

the 5D-ASC scale were significant compared with placebo for both doses and all of the scales, with the exception of the effects of the 200 µg dose on anxiety (Table 1). At 200 µg, LSD produced significant and relevantly higher ratings of blissful state, insightfulness, and changed meaning of percepts compared with 100 µg (one asterisk $p < 0.05$, two asterisks $p < 0.01$, t tests). The data are expressed as the mean \pm SEM in 24 subjects and 16 subjects for the 100 and 200 µg doses of LSD, respectively

LSD produced mean MEQ30 total score ratings of 61 % (range 40–98 %) and a complete mystical experience in only two participants (12.5 %). The MEQ has typically been used with psilocybin, and data on MEQ30 scores are available for various doses of psilocybin, placebo, and methylphenidate (active placebo; Barrett et al. 2015). Psilocybin (at the highest studied dose of 30 mg/70 kg) produced a high mean MEQ30 total score rating of 77 % and complete mystical experiences in as many as 67 % of healthy subjects (Barrett et al. 2015). However, in this psilocybin study setting, inactive and active placebo (methylphenidate) also produced high mean MEQ30 ratings of 23 and 33 %, respectively (Barrett et al. 2015). In contrast, in the present study, placebo increased MEQ30 scores only to 1 %. Similarly, MDMA and methylphenidate produced only small increases in MEQ scores in a similar laboratory setting (Schmid et al. 2014). Another study evaluated psilocybin-assisted psychotherapy in tobacco smokers and also found complete mystical experiences in only 10 of 26 sessions (38 %) that were conducted in 14 patients with high-dose psilocybin (30 mg/70 kg; Garcia-Romeu et al. 2015; Johnson et al. 2014). Accounting for the higher placebo ratings in some of the psilocybin studies compared with our study, LSD increased MEQ30 score differences from placebo overall more than psilocybin and produced greater ineffability and positive mood but lower effects on the mystical subscale than psilocybin (Barrett et al. 2015).

Additionally, the MEQ has been used in patients with anxiety associated with life-threatening illness who were treated with 200 µg LSD (Gasser et al. 2014; Gasser et al. 2015). In

this therapeutic setting, LSD produced similar mystical experiences as in the present study and complete mystical experiences in only two of 11 patients. MEQ scores were only within the range of 3–9 % after active placebo administration (25 µg LSD) on the MEQ subscales. Altogether, these findings indicate that mainly the placebo response and/or the expectancy of a mystical experience were greater in the study setting in some psilocybin studies compared with the LSD studies. Additionally, the participants in the psilocybin studies may have been more spiritually inclined (Griffiths et al. 2006) than our study participants leading to more mystical experiences (Studerus et al. 2012). Furthermore, others may have provided more extensive preparation of the subjects and interpersonal support, contributing to mystical experiences.

The present findings do not support the view that LSD produces lower overall effects than psilocybin at the doses tested. In contrast, the high dose of LSD (200 µg) produced greater placebo-adjusted positive mood ratings than psilocybin on the MEQ30 (Barrett et al. 2015) and very pronounced increases in 5D-ASC blissful state ratings and produced far greater effects than the highest doses of psilocybin or dimethyltryptamine (DMT) that were tested so far on this scale (Gouzoulis-Mayfrank et al. 2005; Hasler et al. 2004). Additionally, LSD-induced MEQ scores were highly correlated with 5D-ASC scores in the present study.

One could argue that mystical and spiritual experiences are not the most prominent feature of the LSD response. Mean ratings on the spiritual experience scale of the 5D-ASC were 22 and 33 % at the 100 and 200 µg doses, respectively, in the

Table 2 Associations between LSD-induced alterations in consciousness (5D-ASC) and mystical experiences (MEQ30)

	Mystical Effects Questionnaire (MEQ30)				
	MEQ30 total score	Mystical	Positive mood	Transcendence of time/space	Ineffability
5D-ASC scale					
Total ASC score	<i>0.87</i>	0.73	0.65	<i>0.82</i>	0.57
Oceanic boundlessness	<i>0.93</i>	<i>0.88</i>	<i>0.83</i>	0.74	0.45
Anxious ego dissolution	0.60	0.39	0.35	0.68	0.55
Visionary restructuralization	0.65	0.54	0.38	0.68	0.45
Auditory alterations	0.30	0.14	0.02	0.49	0.38
Reductions of vigilance	0.61	0.41	0.47	0.64	0.47
Experience of unity	<i>0.82</i>	<i>0.86</i>	<i>0.85</i>	0.56	0.25
Spiritual experience	<i>0.79</i>	<i>0.76</i>	<i>0.76</i>	0.60	0.33
Blissful State	<i>0.80</i>	<i>0.77</i>	<i>0.80</i>	0.72	0.16
Insightfulness	<i>0.77</i>	<i>0.79</i>	0.68	0.52	0.42
Disembodiment	0.71	0.53	0.62	0.71	0.41
Impaired control and cognition	0.63	0.37	0.45	<i>0.79</i>	0.46
Anxiety	0.45	0.32	0.19	0.47	0.51
Complex imagery	0.48	0.31	0.32	0.69	0.19
Elementary imagery	0.36	0.37	0.08	0.29	0.42
Audio-visual synesthesia	0.23	0.07	0.22	0.45	-0.01
Changed meaning of percepts	<i>0.80</i>	0.67	0.59	0.70	0.63
Ego dissolution (item 71)	0.74	0.73	0.74	0.65	0.12

Values are Pearson correlation coefficients in 16 subjects describing correlations between %5D-ASC and %MEQ30 scores. Bold values for $P < 0.05$, italic values for $P < 0.001$

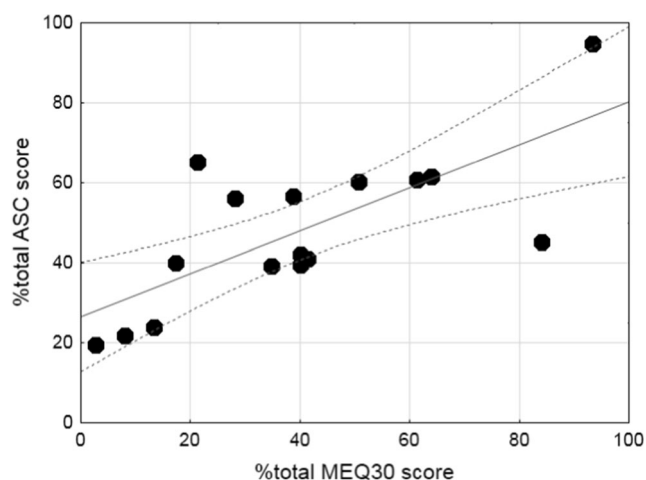


Fig. 3 LSD-induced alterations of consciousness are significantly associated with the LSD-induced mystical experience. The data are expressed as a percentage of ASC total scores on the 5D-ASC scale and a percentage of total scores on the MEQ30 for each of 16 participants after administration of 200 μ g LSD. The lines indicate the regression and 95 % confidence intervals ($R_p = 0.87$, $p < 0.001$)

present study and approximately 23 % after 75 μ g LSD in another study (Carhart-Harris et al. 2016c). Mean ratings of “the experience had a spiritual or mystical quality” were also only approximately 28 % in an imaging study that evaluated the effects of LSD (Tagliazucchi et al. 2016). However, a direct within-subjects comparison of LSD and psilocybin in the same research setting is needed to determine possible differences in mystical-type responses between these substances. Whether mystical-type experiences (Barrett et al. 2015; Garcia-Romeu et al. 2015; MacLean et al. 2011) are critical for the therapeutic potential of substance-assisted psychotherapy requires further study. At least in the case of LSD, the mystical experiences (MEQ scores) were highly associated with other alterations of consciousness on the 5D-ASC scale, and LSD produced additional effects on emotion processing that could facilitate psychotherapeutic interventions (Dolder et al. 2016).

Recent experimental studies associated the subjective effects of LSD (75 μ g, intravenous) on the 5D-ASC scale with fMRI data but in the absence of data on plasma LSD levels

Table 3 Associations between predicted maximal LSD plasma concentrations (C_{\max}) and LSD exposure (AUC) and alterations in consciousness (5D-ASC) and mystical experiences (MEQ30)

	$N = 24$		$N = 16$	
	100 μg		200 μg	
	C_{\max}	AUC	C_{\max}	AUC
5D-ASC scale				
ASC total score	0.19	0.21	-0.35	0.15
Oceanic boundlessness	0.24	0.26	-0.35	0.10
Anxious ego dissolution	0.04	0.07	-0.10	0.32
Visionary restructuration	0.12	0.15	-0.59	-0.16
Auditory alterations	0.02	0.12	-0.18	0.08
Reductions of vigilance	-0.01	0.13	-0.10	0.38
Experience of unity	0.34	0.33	-0.03	0.33
Spiritual experience	-0.02	0.06	-0.32	-0.03
Blissful state	0.25	0.14	-0.23	0.03
Insightfulness	0.24	0.20	-0.37	0.12
Disembodiment	-0.04	0.08	-0.23	0.08
Impaired control and cognition	-0.01	0.01	-0.20	0.18
Anxiety	0.22	0.30	0.01	0.38
Complex imagery	0.06	0.14	-0.28	-0.04
Elementary imagery	-0.13	-0.03	-0.53	-0.15
Audio-visual synesthesia	0.23	0.26	-0.01	0.00
Changed meaning of percepts	-0.03	-0.06	-0.62	-0.10
Ego dissolution (item 71)	0.40	0.51	-0.27	-0.14
MEQ30				
MEQ30 total score	NA		-0.30	0.17
Mystical	NA		-0.25	0.13
Positive mood	NA		-0.08	0.21
Transcendence of time/space	NA		-0.23	0.10
Ineffability	NA		-0.49	0.13

Values are Pearson correlation coefficients describing correlations, the peak concentrations of LSD predicted by the one-compartment model, and LSD-induced %5D-ASC and %MEQ30 scores. Bold values for $P < 0.05C_{\max}$ maximal LSD plasma concentration predicted by the one-compartment pharmacokinetic model, AUC area under the LSD concentration-time curve predicted by the model

(Carhart-Harris et al. 2016c; Kaelen et al. 2016; Lebedev et al. 2016; Roseman et al. 2016). Assuming high oral bioavailability of LSD of 70–100 % (Dolder et al. 2015b), similar plasma exposure (AUC) can be assumed after oral administration of 100 μg LSD (present study I) or intravenous administration of 75 μg LSD (all studies by Carhart-Harris and colleagues). Supporting this assumption, the intravenous 75 μg dose of LSD produced very similar mean ratings on the 5D-ASC scale (Carhart-Harris et al. 2016b) to the present study that used an oral dose of 100 μg . In contrast, the 200 μg dose produced significantly greater ASC total scores and particularly greater 5D-ASC subscale scores of blissful state, insightfulness, and

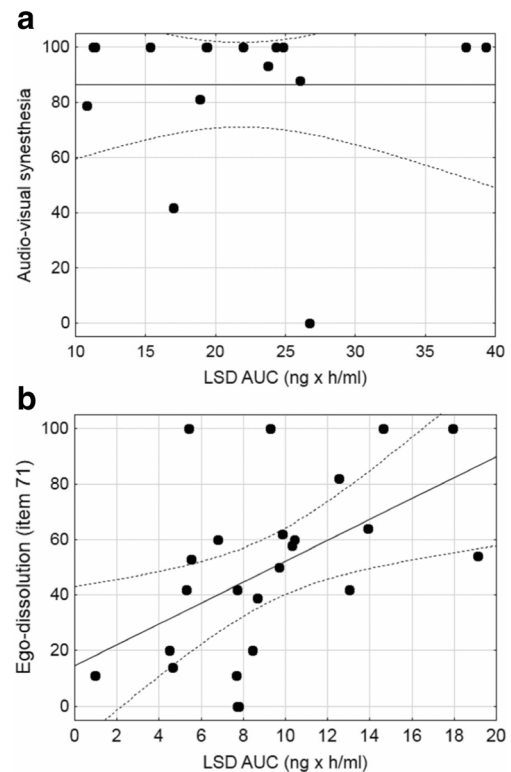


Fig. 4 Correlations between plasma LSD concentrations and subjective peak effects. **a** At 200 μg , LSD induced high ratings of audio-visual synesthesia in all but two of the 16 participants. There was little variance in the response and no correlation between total plasma exposure to LSD (area under the concentration-time curve [AUC]) and audio-visual synesthesia ($R_p = 0.0$, $p > 0.05$, $n = 16$). **b** In contrast, ego dissolution was present to highly variable degrees across subjects after administration of 100 μg LSD. Total exposure to LSD (AUC) positively correlated with LSD-induced ego dissolution ($R_p = 0.51$, $p < 0.05$, $n = 24$). The lines indicate the regression and 95 % confidence intervals

changed meaning of percepts. As previously reported, the 200 μg dose of LSD also produced greater feelings of closeness to others, happiness, openness, and trust than the 100 μg dose (Dolder et al. 2016). Altogether, the data indicate that the 200 μg dose produces overall greater effects and particularly more positive and MDMA-like effects than lower doses (Dolder et al. 2016). This is relevant because the higher dose is currently being used in LSD-assisted psychotherapy (Gasser et al. 2014; Gasser et al. 2015), and the lower dose is being tested in experimental fMRI studies (Carhart-Harris et al. 2016c). The 200 μg dose of LSD also produced greater ASC scores than high doses of the serotonergic hallucinogens DMT and psilocybin (Gouzoulis-Mayfrank et al. 2005; Hasler et al. 2004; Vollenweider and Kometer 2010), ketamine (Gouzoulis-Mayfrank et al. 2005; Studerus et al. 2010), and MDMA (Hysek et al. 2011), although direct comparisons within the same studies and subjects are missing.

The present analyses showed no positive correlations between LSD levels and effects across subjects, possibly because of the relatively high levels of LSD and generally

consistently high subjective response ratings in most subjects. Thus, if relatively high and similar doses of LSD are used that result in plasma levels clearly above the EC_{50} of a particular response measure, then it is unlikely that the response varies relevantly across subjects because responses are close to maximal. This would typically also be the case with measures with a maximal effect limit such as VAS ratings and some physiological effects like pupil size (Hysek and Liechti 2012).

In fact, responses to MDMA or LSD or other drugs in a standardized experimental setting may vary only if the response is not induced consistently in all subjects (e.g., at the beginning of the response) and are mostly attributable to individual differences in drug absorption/distribution (Hysek and Liechti 2012) or when a response is evaluated that is not robustly induced or when a lower dose is used. Specifically, correlations of plasma levels with the subjective and cardiovascular effects of MDMA across subjects are only weak during the peak response but stronger at onset (Hysek and Liechti 2012). This is an important consideration. For example, LSD-induced subjective ego dissolution was recently shown to be associated with specific brain activation patterns in a study that administered a relatively low dose of LSD of 75 μ g intravenously (Tagliazucchi et al. 2016). Interestingly, LSD-induced ego dissolution correlated with plasma LSD levels after administration of an equivalent oral dose of 100 μ g in the present study, and this was the only pharmacodynamic effect of LSD for which a positive association with plasma levels could be demonstrated across subjects. This finding needs to be kept in mind when interpreting associations between ego dissolution and fMRI parameters because the fMRI findings may also reflect other processes that are related to the plasma levels of LSD. Furthermore, the likelihood of detecting correlations within a dose group increases for effects that are not robustly induced in all subjects and thus for effects that are not typically present in all subjects after LSD administration. Finally, unclear is the extent to which a full LSD response was induced in the imaging studies that have been conducted to date because all of these studies used relatively low 75 or 100 μ g doses. In the present study, the 200 μ g dose of LSD produced particularly marked increases in visionary restructuralization including changed meaning of percepts which were significantly greater after the 200 compared with the 100 μ g dose. Contrary to expectations, these perceptual alterations were greater in participants with relatively lower C_{max} levels of LSD within the 200 μ g dose group further supporting the view that higher plasma levels of LSD may not produce greater subjective alterations above a certain threshold level and if high doses of LSD are used.

In conclusion, LSD (200 μ g) rarely produced full mystical experiences in the present study and in patients during LSD-assisted psychotherapy compared with psilocybin in another set and setting. This raises questions regarding expectancy effects and placebo responses and the therapeutic role of

mystical experiences. LSD produced significantly greater bliss, insightfulness, and changes in meaning of percepts at 200 μ g compared with 100 μ g, in addition to the previously reported greater empathogenic effects. This could be relevant for LSD-assisted psychotherapy (200 μ g) and the interpretation of fMRI data (75–100 μ g). Generally, no association was found between plasma LSD levels and its robust effects when analyzed across different subjects and within a dose group. This may have implications for studies that interrelate different effects of LSD, namely fMRI studies.

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Compliance with ethical standards The studies were conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee. All of the subjects provided written consent before participating in either of the studies.

Conflict of interest None.

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References

- Barrett FS, Johnson MW, Griffiths RR (2015) Validation of the revised mystical experience questionnaire in experimental sessions with psilocybin. *J Psychopharmacol* 29:1182–1190
- Baumeister D, Barnes G, Giaroli G, Tracy D (2014) Classical hallucinogens as antidepressants? A review of pharmacodynamics and putative clinical roles. *Ther Adv Psychopharmacol* 4:156–169
- Bogenschutz MP, Forcehimes AA, Pommy JA, Wilcox CE, Barbosa PC, Strassman RJ (2015) Psilocybin-assisted treatment for alcohol dependence: a proof-of-concept study. *J Psychopharmacol* 29:289–299
- Carhart-Harris RL, Kaelen M, Whalley MG, Bolstridge M, Feilding A, Nutt DJ (2015) LSD enhances suggestibility in healthy volunteers. *Psychopharmacology* 232:785–794
- Carhart-Harris RL, Bolstridge M, Rucker J, Day CM, Erritzoe D, Kaelen M, Bloomfield M, Rickard JA, Forbes B, Feilding A, Taylor D, Pilling S, Curran VH, Nutt DJ (2016a) Psilocybin with psychological support for treatment-resistant depression: an open-label feasibility study. *Lancet Psychiatry* 3:619–627
- Carhart-Harris RL, Kaelen M, Bolstridge M, Williams TM, Williams LT, Underwood R, Feilding A, Nutt DJ (2016b) The paradoxical psychological effects of lysergic acid diethylamide (LSD). *Psychol Med* 46:1379–1390
- Carhart-Harris RL, Muthukumaraswamy S, Roseman L, Kaelen M, Droog W, Murphy K, Tagliazucchi E, Schenberg EE, Nest T,

- Orban C, Leech R, Williams LT, Williams TM, Bolstridge M, Sessa B, McGonigle J, Sereno MI, Nichols D, Hellyer PJ, Hobden P, Evans J, Singh KD, Wise RG, Curran HV, Feilding A, Nutt DJ (2016c) Neural correlates of the LSD experience revealed by multi-modal neuroimaging. *Proc Natl Acad Sci U S A* 113:4853–4858
- Davenport WJ (2016) Psychedelic and nonpsychedelic LSD and psilocybin for cluster headache. *CMAJ* 188:217
- Diesch MK (2015) LSD: Rückkehr in the Klinische Forschung. Nachtschatten Verlag, Solothurn
- Dittrich A (1998) The standardized psychometric assessment of altered states of consciousness (ASCs) in humans. *Pharmacopsychiatry* 31(Suppl 2):80–84
- Dolder PC, Liechti ME, Rentsch KM (2015a) Development and validation of a rapid turboflow LC-MS/MS method for the quantification of LSD and 2-oxo-3-hydroxy LSD in serum and urine samples of emergency toxicological cases. *Anal Bioanal Chem* 407:1577–1584
- Dolder PC, Schmid Y, Haschke M, Rentsch KM, Liechti ME (2015b) Pharmacokinetics and concentration-effect relationship of oral LSD in humans. *Int J Neuropsychopharmacol* 19. doi:10.1093/ijnp/pyv072
- Dolder PC, Schmid Y, Mueller F, Borgwardt S, Liechti ME (2016) LSD acutely impairs fear recognition and enhances emotional empathy and sociality. *Neuropsychopharmacology* 41:2638–2646
- Garcia-Romeu A, Griffiths RR, Johnson MW (2015) Psilocybin-occasioned mystical experiences in the treatment of tobacco addiction. *Curr Drug Abuse Rev* 7:157–164
- Gasser P, Holstein D, Michel Y, Doblin R, Yazar-Klosinski B, Passie T, Brenneisen R (2014) Safety and efficacy of lysergic acid diethylamide-assisted psychotherapy for anxiety associated with life-threatening diseases. *J Nerv Ment Dis* 202:513–520
- Gasser P, Kirchner K, Passie T (2015) LSD-assisted psychotherapy for anxiety associated with a life-threatening disease: a qualitative study of acute and sustained subjective effects. *J Psychopharmacol* 29:57–68
- Gouzoulis-Mayfrank E, Heekeren K, Neukirch A, Stoll M, Stock C, Obradovic M, Kovar KA (2005) Psychological effects of (S)-ketamine and N,N-dimethyltryptamine (DMT): a double-blind, cross-over study in healthy volunteers. *Pharmacopsychiatry* 38:301–311
- Griffiths R (2016) Overview of the Johns Hopkins psilocybin research project. Interdisciplinary Conference on Psychedelics Research, Amsterdam, June 3–5, 2016
- Griffiths RR, Richards WA, McCann U, Jesse R (2006) Psilocybin can occasion mystical-type experiences having substantial and sustained personal meaning and spiritual significance. *Psychopharmacology* 187:268–283 discussion 284–292
- Griffiths R, Richards W, Johnson M, McCann U, Jesse R (2008) Mystical-type experiences occasioned by psilocybin mediate the attribution of personal meaning and spiritual significance 14 months later. *J Psychopharmacol* 22:621–632
- Griffiths RR, Johnson MW, Richards WA, Richards BD, McCann U, Jesse R (2011) Psilocybin occasioned mystical-type experiences: immediate and persisting dose-related effects. *Psychopharmacology* 218:649–665
- Grob CS, Danforth AL, Chopra GS, Hagerty M, McKay CR, Halberstadt AL, Greer GR (2011) Pilot study of psilocybin treatment for anxiety in patients with advanced-stage cancer. *Arch Gen Psych* 68:71–78
- Guss J (2016) The NYU School of Medicine study on psilocybin-assisted therapy for treatment of existential distress in cancer patients: history, study structure, therapist training, outcome data. Interdisciplinary Conference on Psychedelic Research Amsterdam 3–5 June, 2016
- Hasler F, Grimberg U, Benz MA, Huber T, Vollenweider FX (2004) Acute psychological and physiological effects of psilocybin in healthy humans: a double-blind, placebo-controlled dose-effect study. *Psychopharmacology* 172:145–156
- Hysek CM, Liechti ME (2012) Effects of MDMA alone and after pretreatment with reboxetine, duloxetine, clonidine, carvedilol, and doxazosin on pupillary light reflex. *Psychopharmacology* 224:363–376
- Hysek CM, Simmler LD, Ineichen M, Grouzmann E, Hoener MC, Brenneisen R, Huwyler J, Liechti ME (2011) The norepinephrine transporter inhibitor reboxetine reduces stimulant effects of MDMA ("ecstasy") in humans. *Clin Pharmacol Ther* 90:246–255
- Johnson MW, Garcia-Romeu A, Cosimano MP, Griffiths RR (2014) Pilot study of the 5-HT_{2A}R agonist psilocybin in the treatment of tobacco addiction. *J Psychopharmacol* 28:983–992
- Kaelen M, Barrett FS, Roseman L, Lorenz R, Family N, Bolstridge M, Curran HV, Feilding A, Nutt DJ, Carhart-Harris RL (2015) LSD enhances the emotional response to music. *Psychopharmacology* 232:3607–3614
- Kaelen M, Roseman L, Kahan J, Santos-Ribeiro A, Orban C, Lorenz R, Barrett FS, Bolstridge M, Williams T, Williams L, Wall MB, Feilding A, Muthukumaraswamy S, Nutt DJ, Carhart-Harris R (2016) LSD modulates music-induced imagery via changes in parahippocampal connectivity. *Eur Neuropsychopharmacol* 26:1099–1109
- Krebs TS, Johansen PO (2012) Lysergic acid diethylamide (LSD) for alcoholism: meta-analysis of randomized controlled trials. *J Psychopharmacol* 26:994–1002
- Krebs TS, Johansen PO (2013) Over 30 million psychedelic users in the United States. *F1000Res* 2:98
- Kupferschmidt K (2014) High hopes. *Science* 345:18–23
- Lebedev AV, Kaelen M, Lovden M, Nilsson J, Feilding A, Nutt DJ, Carhart-Harris RL (2016) LSD-induced entropic brain activity predicts subsequent personality change. *Hum Brain Mapp* 37:3203–3213
- MacLean KA, Johnson MW, Griffiths RR (2011) Mystical experiences occasioned by the hallucinogen psilocybin lead to increases in the personality domain of openness. *J Psychopharmacol* 25:1453–1461
- MacLean KA, Leoutsakos JM, Johnson MW, Griffiths RR (2012) Factor analysis of the Mystical Experience Questionnaire: a study of experiences occasioned by the hallucinogen psilocybin. *J Sci Study Relig* 51:721–737
- Nichols DE (2016) Psychedelics. *Pharmacol Rev* 68:264–355
- Pahnke WN (1969) Psychedelic drugs and mystical experience. *Int Psychiatry Clin* 5:149–162
- Pahnke WN, Kurland AA, Unger S, Savage C, Grof S (1970) The experimental use of psychedelic (LSD) psychotherapy. *JAMA* 212:1856–1863
- Passie T, Halpern JH, Stichtenoth DO, Emrich HM, Hintzen A (2008) The pharmacology of lysergic acid diethylamide: a review. *CNS Neurosci Ther* 14:295–314
- Rickli A, Moning OD, Hoener MC, Liechti ME (2016) Receptor interaction profiles of novel psychoactive tryptamines compared with classic hallucinogens. *Eur Neuropsychopharmacol* 26:1327–1337
- Roseman L, Sereno MI, Leech R, Kaelen M, Orban C, McGonigle J, Feilding A, Nutt DJ, Carhart-Harris RL (2016) LSD alters eyes-closed functional connectivity within the early visual cortex in a retinotopic fashion. *Hum Brain Mapp* 37:3031–3040
- Schmid Y, Hysek CM, Simmler LD, Crockett MJ, Quednow BB, Liechti ME (2014) Differential effects of MDMA and methylphenidate on social cognition. *J Psychopharmacol* 28:847–856
- Schmid Y, Enzler F, Gasser P, Grouzmann E, Preller KH, Vollenweider FX, Brenneisen R, Muller F, Borgwardt S, Liechti ME (2015) Acute effects of lysergic acid diethylamide in healthy subjects. *Biol Psychiatry* 78:544–553
- Speth J, Speth C, Kaelen M, Schloerscheidt AM, Feilding A, Nutt DJ, Carhart-Harris RL (2016) Decreased mental time travel to the past correlates with default-mode network disintegration under lysergic acid diethylamide. *J Psychopharmacol* 30:344–353
- Steuer AE, Poetzsch M, Stock L, Eisenbeiss L, Schmid Y, Liechti ME, Kraemer T (2016) Development and validation of an ultra-fast and sensitive microflow liquid chromatography-tandem mass spectrometry (MFLC-MS/MS) method for quantification of LSD and its

- metabolites in plasma and application to a controlled LSD administration study in humans. *Drug Test Anal.* doi:10.1002/dta.2042
- Strajhar P, Schmid Y, Liakoni E, Dolder PC, Rentsch KM, Kratschmar DV, Odermatt A, Liechti ME (2016) Acute effects of lysergic acid diethylamide on circulating steroid levels in healthy subjects. *J Neuroendocrinol* 28:12374
- Studerus E, Gamma A, Vollenweider FX (2010) Psychometric evaluation of the altered states of consciousness rating scale (OAV). *PLoS One* 5:e12412
- Studerus E, Gamma A, Komater M, Vollenweider FX (2012) Prediction of psilocybin response in healthy volunteers. *PLoS One* 7:e30800
- Tagliazucchi E, Roseman L, Kaelen M, Orban C, Muthukumaraswamy SD, Murphy K, Laufs H, Leech R, McGonigle J, Crossley N, Bullmore E, Williams T, Bolstridge M, Feilding A, Nutt DJ, Carhart-Harris R (2016) Increased global functional connectivity correlates with LSD-induced ego dissolution. *Curr Biol* 26:1043–1050
- Terhune DB, Luke DP, Kaelen M, Bolstridge M, Feilding A, Nutt D, Carhart-Harris R, Ward J (2016) A placebo-controlled investigation of synaesthesia-like experiences under LSD. *Neuropsychologia* 88:28–34
- Turek IS, Soskin RA, Kurland AA (1974) Methylenedioxyamphetamine (MDA)-subjective effects. *J Psychoactive Drugs* 6:7–14
- Vollenweider FX, Komater M (2010) The neurobiology of psychedelic drugs: implications for the treatment of mood disorders. *Nat Rev Neurosci* 11:642–651
- Vollenweider FX, Csomor PA, Knappe B, Geyer MA, Quednow BB (2007) The effects of the preferential 5-HT_{2A} agonist psilocybin on prepulse inhibition of startle in healthy human volunteers depend on interstimulus interval. *Neuropsychopharmacology* 32:1876–1887

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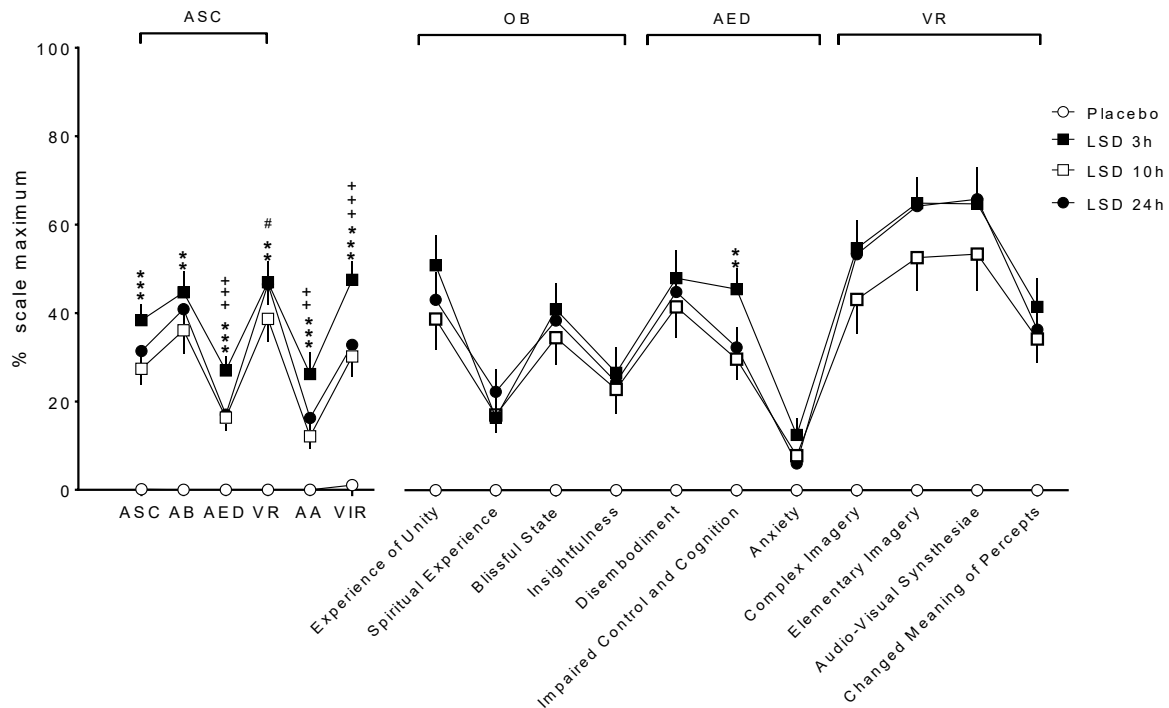


Figure S1. Effects of LSD (100 µg) on the 5 Dimensions of Altered States of Consciousness (5D-ASC) scale repeatedly used at 3, 10, and 24h to retrospectively rate the LSD effects in 24 subjects in Study 1. The aim of the repeated administration was to test whether self-ratings shortly after the peak response (3h), at the end of the response (10h) or on the next day (at 24h) differ from each other. We hypothesized that there would not be any relevant differences. ANOVA with time as between-subject factor (3, 10, and 24 h) on the total ASC score showed a significant effect of time ($F_{2,46}=5.50$, $P>0.01$). Tukey post hoc tests showed higher ratings at 3h compared with 10 and 24h (both $P<0.01$) but no differences between the 10 and 24h ratings. ANOVA with time and dimension (5 main dimensions) as factors showed a significant main effect of time ($F_{2,46}=6.17$, $P<0.01$) and scale $F_{4,92}=19.87$, $P<0.001$ and a significant time and dimension interaction ($F_{8,184}=3.5$, $P<0.001$). Tukey post hoc test showed greater ratings at 3h compared with ratings at 10 h on all dimensions (all $P<0.01$, Figure S1) and compared with ratings at 24 h for AED, AA, and VIR (all $P<0.01$). Ratings at 10 h did not differ from ratings at 24h with the exception of ratings for VR which were greater at 24 h compared with 10 h ($P<0.05$). ANOVA with time and scale (all 11 scales of the 5D-ASC) showed no significant main effect of time ($F_{2,46}=2.37$, $P=0.10$), a significant main effect of scale ($F_{10,230}=18.90$, $P<0.001$) and a significant time and scale interaction ($F_{20,460}=1.81$, $P<0.05$). Post hoc tests showed that only ratings of “impaired control and cognition” were higher at 3h compared with 24h. There were no other differences between the ratings at 3, 10, and 24 h. Together the data indicates higher ratings of the overall effect when assessed during the response at 3h compared to ratings taken immediately after the response or on the next day. However, the differences were minimal and not present between ratings at 10 h and 24 h. OB, oceanic boundlessness; AED, anxious ego-dissolution; VR, visionary restructuralization; AA, auditory alterations; VIR, vigilance reduction. ** $P<0.01$ and *** $P<0.001$ for 3h vs. 10h; ** $P<0.01$ and *** $P<0.001$ for 3h vs. 24h; # $P<0.05$ for 10h vs. 24h (Tukey post hoc tests based on significant time and scale interactions in the ANOVA). The data are expressed as the mean \pm SEM in 24 subjects.

3.4 Publication 4

LSD Acutely Impairs Fear Recognition and Enhances Emotional Empathy and Sociality

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Lysergic acid diethylamide (LSD) is used recreationally and has been evaluated as an adjunct to psychotherapy to treat anxiety in patients with life-threatening illness. LSD is well-known to induce perceptual alterations, but unknown is whether LSD alters emotional processing in ways that can support psychotherapy. We investigated the acute effects of LSD on emotional processing using the Face Emotion Recognition Task (FERT) and Multifaceted Empathy Test (MET). The effects of LSD on social behavior were tested using the Social Value Orientation (SVO) test. Two similar placebo-controlled, double-blind, random-order, crossover studies were conducted using 100 µg LSD in 24 subjects and 200 µg LSD in 16 subjects. All of the subjects were healthy and mostly hallucinogen-naïve 25- to 65-year-old volunteers (20 men, 20 women). LSD produced feelings of happiness, trust, closeness to others, enhanced explicit and implicit emotional empathy on the MET, and impaired the recognition of sad and fearful faces on the FERT. LSD enhanced the participants' desire to be with other people and increased their prosocial behavior on the SVO test. These effects of LSD on emotion processing and sociality may be useful for LSD-assisted psychotherapy.

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INTRODUCTION

The classic serotonergic psychedelic/hallucinogen lysergic acid diethylamide (LSD) was widely studied in humans in the 1950s to 1970s. However, little to no clinical research on LSD has been conducted since then (Nichols, 2016; Passie *et al*, 2008). Today, LSD is again the focus of clinical investigations, including experimental studies in healthy subjects (Carhart-Harris *et al*, 2016, 2015; Dolder *et al*, 2015b; Schmid *et al*, 2015; Strajhar *et al*, 2016), and clinical trials that evaluate LSD-assisted psychotherapy (Gasser *et al*, 2014). LSD that was administered only a few times decreased anxiety and increased quality of life over a period of 12 months in patients with anxiety associated with terminal illness (Gasser *et al*, 2015). The acute LSD experiences were hypothesized to lead to a restructuring of the person's emotional trust and situational understanding (Gasser *et al*, 2015). Similar to LSD, the serotonergic hallucinogen psilocybin and serotonin (5-hydroxytryptamine (5-HT)) releaser 3,4-methylenedioxymethamphetamine (MDMA; ecstasy) have been used to facilitate psychotherapy in clinical trials (Grob *et al*, 2011; Mithoefer *et al*, 2010; Oehen *et al*, 2013). Psilocybin reduced anxiety at 3 months

and additionally improved mood at 6 months after treatment in patients with advanced-stage cancer (Grob *et al*, 2011). Additionally, psilocybin was recently studied as a treatment for tobacco (Johnson *et al*, 2014) and alcohol (Bogenschutz *et al*, 2015) dependence. MDMA-assisted psychotherapy reduced symptoms of post-traumatic stress disorder at 2 months (Mithoefer *et al*, 2010), and the benefits of MDMA were reportedly sustained for several years (Mithoefer *et al*, 2013). These first findings from modern clinical studies with psychedelics and MDMA should be confirmed in larger trials. Exploring the mechanisms that may contribute to these beneficial and lasting effects after only a few administrations of the substances is also important.

Studies that use psychedelics and MDMA in healthy subjects are well suited to assess the mechanism of action of these substances. Both LSD and psilocybin appear to produce effects that last beyond the acute drug response in both patients and healthy subjects. Specifically, LSD increased optimism and trait openness at 2 weeks (Carhart-Harris *et al*, 2016), and psilocybin produced positive changes in attitudes, mood, and behavior at 2 (Griffiths *et al*, 2006) and 14 months (Griffiths *et al*, 2011) after administration. Psilocybin increased personality trait openness in participants who had 'mystical experiences' during their psilocybin session (MacLean *et al*, 2011). Therefore, some of the lasting beneficial effects appear to be associated with an acute psychedelic response, including a 'peak' or 'mystical' experience (Carhart-Harris *et al*, 2016; MacLean *et al*, 2011).

Both LSD and psilocybin are 5-HT_{2A} receptor agonists, and their psychedelic effects are mediated by 5-HT_{2A}

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receptor stimulation (Vollenweider *et al*, 1998). The long-term effects of LSD and psilocybin may be related to their psychedelic and 5-HT_{2A} receptor activation properties. In contrast to the psychedelics LSD and psilocybin, MDMA is considered an empathogen (entactogen) that mainly enhances positive feelings, empathy, and prosociality (Hysek *et al*, 2014a; Kirkpatrick *et al*, 2014) while having few hallucinogen-like effects. Additionally, MDMA has been shown to positively alter emotion processing (Bedi *et al*, 2010; Hysek *et al*, 2012, 2014a; Kirkpatrick *et al*, 2012, 2014; Schmid *et al*, 2014). These acute effects of MDMA on emotion processing and social behavior may be beneficial during psychotherapy in the absence of a full psychedelic peak experience. LSD also produced acute MDMA-like subjective effects, including greater well-being, happiness, closeness to others, openness, and trust (Schmid *et al*, 2015). Thus, LSD and MDMA may have common effects on the processing of emotional information with relevance to their positive acute and possibly long-term effects during psychotherapy. However, the effects of LSD in tests of emotion processing are unknown. Therefore, the present study investigated the acute effects of LSD using the Face Emotion Recognition Task (FERT) and Multifaceted Empathy Test (MET). The effects of LSD on social behavior were also evaluated using the Social Value Orientation (SVO) test. Additionally, we assessed the subjective mood effects of LSD using Visual Analog Scales (VASs) and the Adjective Mood Rating Scale (AMRS), vital signs, and adverse effects. We hypothesized that LSD would impair the recognition of negative emotions on the FERT and enhance emotional empathy on the MET and prosociality on the SVO test.

MATERIALS AND METHODS

Study Design

We pooled data from two similar studies using double-blind, placebo-controlled, crossover designs with two experimental test sessions (LSD and placebo) in a balanced order. Study 1 used a dose of 100 µg LSD and placebo in 24 subjects. Study 2 used 200 µg LSD or placebo in 16 subjects. The washout periods between sessions were at least 7 days. The studies were conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee. The administration of LSD to healthy subjects was authorized by the Swiss Federal Office for Public Health, Bern, Switzerland. All of the subjects provided written consent before participating in either of the studies, and they were paid for their participation. The studies were registered at ClinicalTrials.gov (NCT02308969, NCT01878942). The subjective, endocrine, and pharmacokinetic effects of LSD in Study 2 were previously reported (Dolder *et al*, 2015b; Schmid *et al*, 2015; Strajhar *et al*, 2016).

Participants

Forty healthy participants were recruited from the University of Basel campus via online advertisement. Twenty-four subjects (12 men, 12 women; 33 ± 11 years old (mean ± SD); range, 25–60 years) participated in Study 1, and 16 subjects (8 men, 8 women; 29 ± 6 years old; range, 25–51 years) participated in Study 2. The inclusion and exclusion criteria

were identical for both studies. Subjects younger than 25 years of age were excluded from participating in the study. Additional exclusion criteria were age > 65 years, pregnancy (urine pregnancy test at screening and before each test session), personal or family (first-degree relative) history of major psychiatric disorders (assessed by the semistructured clinical interview for *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition, Axis I disorders by the study physician and an additional interview by a trained psychiatrist), use of medications that may interfere with the study medication, chronic or acute physical illness (abnormal physical exam, electrocardiogram, or hematological and chemical blood analyses), tobacco smoking (> 10 cigarettes/day), lifetime prevalence of illicit drug use > 10 times (except for tetrahydrocannabinol), illicit drug use within the last 2 months, and illicit drug use during the study (determined by urine drug tests). The subjects were asked to abstain from excessive alcohol consumption between test sessions and particularly limit their use to one standard drink on the day before the test sessions. Additionally, the participants were not allowed to drink xanthine-containing liquids after midnight before the study day. Eleven subjects had used a hallucinogen including LSD (6 participants) one to three times, and most of the subjects (29) were hallucinogen-naïve (Supplementary Table S1). We performed urine drug tests at screening and before each test session, and no substances were detected during the study.

Study Procedures

Each study included a screening visit, a psychiatric interview, two 25-h experimental sessions, and an end-of-study visit. The experimental sessions were conducted in a quiet standard hospital patient room. The participants were resting in hospital beds except when going to the restroom. Only one research subject and one investigator were present during the experimental sessions. Participants could interact with the investigator, rest quietly and/or listen to music via headphones, but no other entertainment was provided. LSD or placebo was administered at 0900 hours. The subjects were never alone during the first 12 h after drug administration, and the investigator was in a room next to the subject for up to 24 h while subjects were asleep (mostly from 0100 to 0800 hours). Because subjective responses to LSD are pronounced and peak at 2–3 h and last up to 12 h (Passie *et al*, 2008; Schmid *et al*, 2015), effects on emotion processing and prosociality were assessed 5 and 7 h after the 100 and 200 µg doses, respectively, when the subjective effects of LSD amounted to approximately 50% of the peak responses (Dolder *et al*, 2015b; Schmid *et al*, 2015).

Study Drug

LSD (D-LSD hydrate; Lipomed AG, Arlesheim, Switzerland) was administered in single oral doses of 100 or 200 µg. Both doses are within the range of doses that are taken for recreational purposes (Passie *et al*, 2008).

Measures

Facial Emotion Recognition Task. We used the FERT, which is sensitive to the effects of other psychoactive

substances, including serotonin and norepinephrine uptake inhibitors (Harmer *et al*, 2004), MDMA (Bedi *et al*, 2010; Hysek *et al*, 2014b; Kirkpatrick *et al*, 2014; Schmid *et al*, 2014), and methylphenidate (Hysek *et al*, 2014b; Schmid *et al*, 2014). The task included 10 neutral faces and 160 faces that expressed one of four basic emotions (ie, happiness, sadness, anger, and fear), with pictures morphed between 0% (neutral) and 100% in 10% steps. Two female and two male pictures were used for each of the four emotions. The stimuli were presented in random order for 500 ms and then were replaced by the rating screen where participants had to indicate the correct emotion. The outcome measure was accuracy (proportion correct). The FERT was performed 5 and 7 h after the 100 and 200 µg doses of LSD, respectively.

Multifaceted Empathy Test. The MET is a reliable and valid task that assesses the cognitive and emotional aspects of empathy (Dziobek *et al*, 2008). The MET has been shown to be sensitive to oxytocin (Hurlemann *et al*, 2010), MDMA (Hysek *et al*, 2014a; Kuypers *et al*, 2014; Schmid *et al*, 2014), and psilocybin (Preller *et al*, 2015). The computer-assisted test consisted of 40 photographs that showed people in emotionally charged situations. To assess cognitive empathy, the participants were required to infer the mental state of the subject in each scene and indicate the correct mental state from a list of four responses. Cognitive empathy was defined as the percentage of correct responses relative to total responses. To measure emotional empathy, the subjects were asked to rate how much they were feeling for an individual in each scene (ie, explicit emotional empathy) and how much they were aroused by each scene (ie, implicit emotional empathy) on a 1–9 point scale. The latter rating provides an inherent additional assessment of emotional empathy, which is considered to reduce the likelihood of socially desirable answers. The three aspects of empathy were each tested with 20 stimuli with positive valence and 20 stimuli with negative valence, resulting in a total of 120 trials. The MET was performed 5 h and 30 min after the 100 µg LSD dose and 7 h and 30 min after the 200 µg LSD, respectively.

SVO test. We used the paper version of the validated SVO test to assess social behavior (Murphy *et al*, 2011). The SVO measure was previously shown to be sensitive to MDMA (Hysek *et al*, 2014a). In this economic resource allocation task, prosociality is defined as behavior that maximizes the sum of resources for the self and others and minimizes the difference between the two. The test consists of six primary and nine secondary SVO slider items with a resource allocation choice over a defined continuum of joint payoffs (Murphy *et al*, 2011). The participants were instructed to choose a resource allocation that defined their most preferred joint distribution between themselves and another person. The allocated funds had real value, and four randomly selected subjects received the funds they earned. Mean allocations for the self and the other were calculated (Hysek *et al*, 2014a; Murphy *et al*, 2011), and the inverse tangent of the ratio of these two means produced an angle that indicated the participants' SVO index. A smaller SVO angle indicates more individualistic or competitive behavior, and a larger SVO angle indicates more prosocial or even

altruistic behavior. The SVO was performed 6 and 8 h after the 100 and 200 µg doses of LSD, respectively.

Subjective mood. The VASs and the AMRS (Janke and Debus, 1978) were repeatedly used to assess subjective effects including aspects of empathy and sociality (Hysek *et al*, 2014a; Schmid *et al*, 2015) (Supplementary Material and Methods).

Vital signs and adverse effects. Blood pressure, heart rate, body temperature, pupil diameter, and adverse effects were measured as described in the Supplementary Material and Methods.

Drug concentrations. Blood samples for the analysis of plasma LSD levels were collected in lithium heparin tubes after completing the social cognitive tests 6 and 8 h after administration of the 100 and 200 µg doses of LSD or placebo, respectively. Plasma LSD concentrations were determined using liquid-chromatography tandem mass spectrometry (Dolder *et al*, 2015a).

Statistical Analyses

All of the data were analyzed using repeated measures analysis of variance (ANOVA), with drug (LSD vs placebo) as the within-subjects factor and dose (100 vs 200 µg) as the between-subjects factor, followed by the Tukey's *post hoc* test based on significant main effects or interactions. Repeated subjective measures were expressed as peak effects prior to the ANOVAs. Additionally, differences at individual time points were also compared using paired *t*-tests. Modulatory effects by sex or previous hallucinogen use were excluded by adding sex or substance use as an additional factor to the ANOVAs. Sex or previous substance use did not moderate outcome measures.

RESULTS

Facial Emotion Recognition

The effects of LSD on the FERT are shown in Figure 1. Data were missing from 2 of the 24 subjects in the 100 µg LSD dose group because of technical problems. LSD impaired the recognition of fearful faces (main effect of drug: $F_{1,36} = 20.71$, $p < 0.001$), with no drug \times dose interaction. Impairments were found in both the 100 and 200 µg dose groups compared with placebo ($p < 0.01$ and $p < 0.05$, respectively). A significant main effect of drug ($F_{1,36} = 7.36$, $p = 0.01$) indicated that LSD also impaired the recognition of sad faces, but *post hoc* comparisons of the two dose groups with placebo did not reach significance. No significant effects of LSD on the decoding of neutral, happy, or angry facial expressions were found.

Empathy

The effects of LSD on explicit emotional and cognitive empathy are shown in Figure 2. Data were missing from 2 of the 24 subjects in the 100 µg LSD dose group because of technical problems. There were significant main effects of drug on explicit and implicit emotional empathy ratings

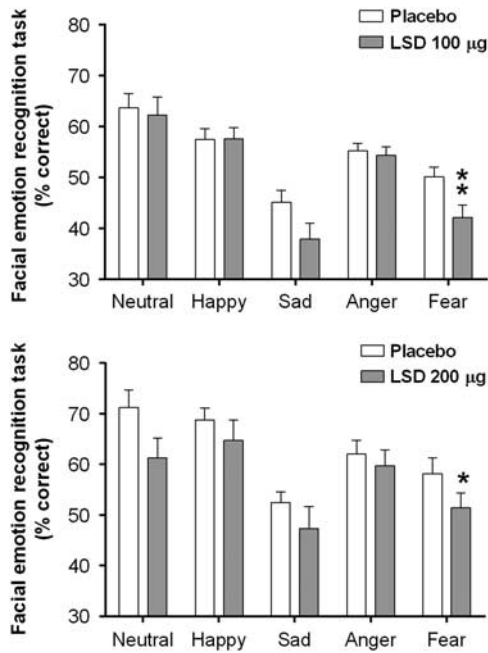


Figure 1 Lysergic acid diethylamide (LSD) impaired fear recognition on the Face Emotion Recognition Task. LSD also impaired the decoding of sad faces (significant main effect of drug), but the effects did not reach statistical significance in the individual dose groups. The data are expressed as mean \pm SEM in 22 and 16 subjects in the 100 and 200 μ g LSD dose groups, respectively. * $p < 0.05$, ** $p < 0.01$, significant difference from placebo.

($F_{1,36} = 14.05$, $p < 0.001$ and $F_{1,36} = 6.71$, $p = 0.01$, respectively), indicating that LSD increased both aspects of emotional empathy. The *post hoc* tests showed that the 200 μ g dose but not the 100 μ g dose of LSD produced a significant effect on explicit ($p < 0.01$) and implicit ($p = 0.01$) empathy scores compared with placebo. The valence-specific analysis showed that LSD significantly increased explicit and implicit emotional empathy scores for positive emotional stimuli ($F_{1,36} = 24.32$, $p < 0.001$ and $F_{1,36} = 10.47$, $p < 0.01$, respectively) but there were only trend effects for negative emotional stimuli ($F_{1,36} = 3.29$, $p = 0.08$ and $F_{1,36} = 2.82$, $p = 0.1$, respectively). LSD decreased cognitive empathy, reflected by a significant main effect of drug ($F_{1,36} = 16.87$, $p < 0.001$). The *post hoc* tests showed that this effect was significant for both the 100 and 200 μ g doses compared with the respective placebo conditions (both $p < 0.05$).

Social Value Orientation

A significant effect of drug was found on the SVO angle ($F_{1,38} = 4.31$, $p < 0.05$), indicating that LSD increased prosociality. The *post hoc* tests showed that this effect did not reach significance in the individual LSD dose groups and was only evident in the larger total study sample.

Subjective Mood Effects

Subjective effects on the VASs are shown in Figure 3, and maximal values are presented in Table 1. LSD increased maximal VAS rating scores, including those reflecting empathy and prosociality such as 'feeling close to others', 'open', 'trust', and 'I want to be with others', with greater

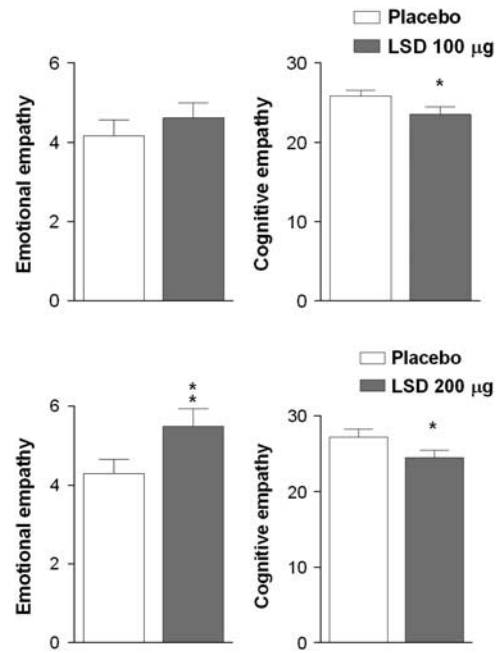


Figure 2 Lysergic acid diethylamide (LSD) increased emotional empathy and decreased cognitive empathy on the Multifaceted Empathy Test. The data are expressed as mean \pm SEM in 22 and 16 subjects in the 100 and 200 μ g LSD dose groups, respectively. * $p < 0.05$, ** $p < 0.01$, significant difference from placebo.

peak effects at the higher compared with the lower dose. Ratings of 'happy' were similarly increased by both doses. LSD produced small dose-dependent increases in 'bad drug effect' and 'fear' (Figure 3, Table 1). On the AMRS, LSD significantly increased ratings of 'well-being', 'emotional excitement', 'inactivity', 'introversion', and 'dreaminess' compared with placebo (Figure 4 and Table 1). There was a significant main effect of LSD on 'fear' but no significant effects in the individual studies.

Vital Signs and Adverse Effects

Peak values and statistics are shown in Table 1. Compared with placebo, LSD increased blood pressure, heart rate, and body temperature as well as pupil size in the dark and after a light stimulus (Table 1). These effects were similar for both doses (no drug \times dose interaction). Compared with placebo, both doses of LSD increased the total acute (0–10 h) adverse effects. Only the high dose increased the total subacute (10–24 h) adverse effects. Adverse effects 24–72 h were slightly increased in the total sample but not in the individual studies (Table 1). The frequently reported adverse effects are presented in Supplementary Table S2. There were no severe adverse events.

Plasma Drug Levels and Correlations Between Effects

Plasma concentrations of LSD were 0.7 ± 0.3 ng/ml (mean \pm SD) 6 h after administration of the 100 μ g dose and 1.3 ± 0.6 ng/ml 8 h after administration of the 200 μ g dose. These time points of blood sample collection were immediately after the social cognitive tests performed in the respective dose groups. Plasma

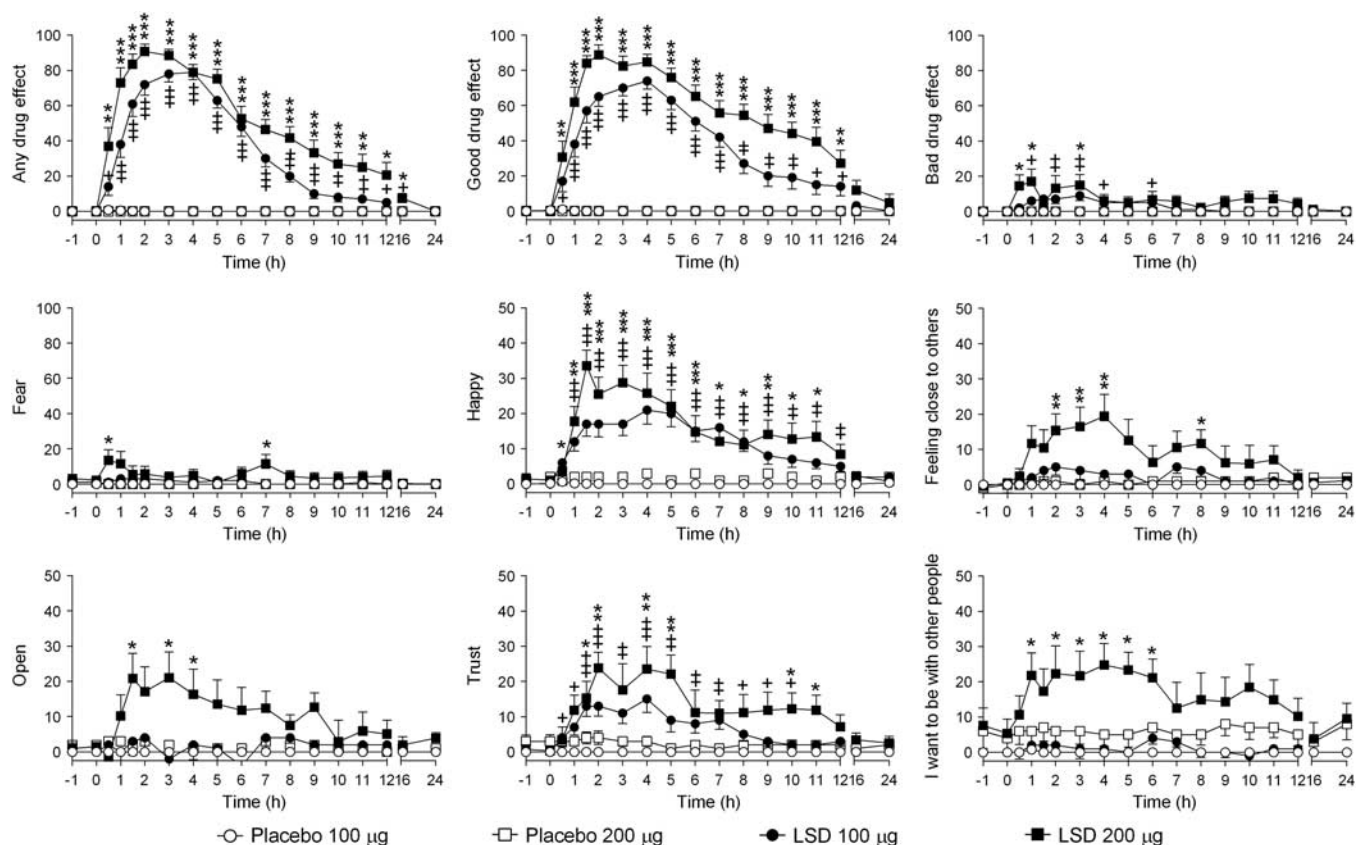


Figure 3 Subjective effects of lysergic acid diethylamide (LSD) over time on the Visual Analog Scales (VASs). LSD or placebo was administered at $t=0$. The data are expressed as mean \pm SEM in 24 and 16 subjects in the 100 and 200 μ g LSD dose groups, respectively. LSD significantly increased ratings on all VASs with significant dose-response effects, except for ratings of 'happy'. The corresponding maximal effects and statistics are shown in Table 1. Emotion recognition (Face Emotion Recognition Task), empathy (Multifaceted Empathy Test), and social value orientation (SVO) tests were conducted 5–6 and 7–8 h after the administration of the 100 and 200 μ g LSD dose, respectively. +/* $p < 0.05$, ++/** $p < 0.01$, +++/** $p < 0.001$ for the 100/200 μ g LSD dose, respectively, compared with placebo (T -tests).

LSD levels correlated with explicit emotional empathy scores on the MET for positive (Spearman $R_s = 0.37$, $p < 0.05$, $n = 38$) but not for negative emotional situations. Plasma levels of LSD were not associated with FERT or SVO test measures. Plasma levels of LSD were associated with LSD-induced ratings of trust (Spearman $R_s = 0.32$, $p < 0.05$, $n = 40$). LSD-induced VAS ratings for feelings of 'closeness' and 'trust' were associated with greater explicit empathy for positive emotional stimuli (Spearman $R_s = 0.35$, $p < 0.05$ and $R_s = 0.47$, $p < 0.01$, respectively, $n = 38$).

DISCUSSION

LSD positively altered the processing of emotional information by decreasing the recognition of fearful and sad faces and enhancing emotional empathy and prosociality. We are aware of no other published data on the acute effects of LSD on emotion processing. However, MDMA produced very similar effects to those of LSD in the present study. MDMA reduced the recognition of sad and fearful faces but not happy faces on the FERT (Bedi *et al*, 2010; Hysek *et al*, 2014b), increased explicit and implicit emotional empathy on the MET (Hysek *et al*, 2014a; Kuypers *et al*, 2014) (mainly for positive emotionally charged situations) (Hysek *et al*, 2014a;

Schmid *et al*, 2014), and increased prosociality on the SVO test (Hysek *et al*, 2014a). LSD did not facilitate perception of happiness in the FERT similar to MDMA (Bedi *et al*, 2010; Hysek *et al*, 2014b), possibly because detection of positive basic emotions is very accurate in healthy subjects and difficult to enhance. Thus, the 5-HT_{2A} receptor agonist LSD and 5-HT releaser MDMA may produce overall similar effects on the processing of emotional information. However, in contrast to MDMA, LSD also impaired cognitive empathy on the MET, and the higher dose also decreased the recognition of neutral faces on the FERT, indicating nonspecific performance effects. Similar to LSD, the 5-HT_{2A} receptor agonist psilocybin decreased the recognition of negative facial expressions (Komter *et al*, 2012) and increased emotional empathy on the MET (Preller *et al*, 2015). Altogether, these findings indicate that LSD affects emotion processing similarly to MDMA and psilocybin.

The marked acute psychedelic/hallucinogenic 'peak response' to LSD and psilocybin has been considered relevant to their lasting effects (Carhart-Harris *et al*, 2016; Griffiths *et al*, 2011). The present study showed that LSD has dose-dependent subjective effects on empathogenic mood, including 'feelings of closeness to others', 'wanting to be with others', 'happiness,' 'openness,' and 'trust' (Schmid *et al*, 2015), in addition to more hallucinogen-specific

Table 1 Values and Statistics for the Subjective and Cardiovascular Peak Effects

	Placebo 100 µg (mean ± SE)	LSD 100 µg (mean ± SE)	Placebo 200 µg (mean ± SE)	LSD 200 µg (mean ± SE)	Drug		Drug × Dose	
					F _{1,38} =	p=	F _{1,38} =	p=
Subjective effects								
Visual Analog Scales (VAS, %)								
Any drug effect	0.9 ± 0.6	87.5 ± 3.3***	0.1 ± 0.1	97.2 ± 1.7***#	1939	***	6.21	*
Good drug effect	0.9 ± 0.6	85.2 ± 3.4***	0.1 ± 0.1	96.8 ± 1.5***##	1661	***	7.66	**
Bad drug effect	0.0 ± 0.0	17.3 ± 3.6**	0.1 ± 0.06	40.0 ± 8.2***###	51.17	***	8.01	**
Fear	0.0 ± 0.0	8.4 ± 2.3	0.06 ± 0.1	31.3 ± 8.6***###	27.74	***	9.24	**
Happy	1.2 ± 0.6	30 ± 3.4***	5.0 ± 2.0	39.1 ± 4.2***	141.5	***	1	NS
Closeness to others	0.0 ± 0.0	15.2 ± 3.2***	4.3 ± 1.8	32.3 ± 4.7***###	61.68	***	5.38	*
Open	0.2 ± 0.2	17.0 ± 2.8***	3.9 ± 1.5	41.0 ± 3.6***###	128.9	***	18.4	***
Trust	0.0 ± 0.0	22.0 ± 4.1***	4.8 ± 2.1	39.8 ± 4.0***###	81.37	***	4.2	*
I want to be hugged	0.0 ± 0.0	8.8 ± 2.7	3.4 ± 1.9	27.8 ± 6.8***###	23.52	***	5.13	*
I want to hug someone	0.0 ± 0.0	10.4 ± 2.7*	− 1.4 ± 3.3	27.6 ± 6.1***##	41.21	***	9.13	**
I want to be alone	0.6 ± 0.6	7.7 ± 2.5	5.1 ± 1.9	17.6 ± 5.6	9.93	**	0.76	NS
I want to be with other people	0.8 ± 0.8	12.8 ± 2.5**	10.8 ± 4.2	42.8 ± 5.5***###	79.87	***	16.25	***
Adjective Mood Rating Scale (AMRS, Δ score)								
Well-being	0.0 ± 0.6	2.5 ± 1.0	1.8 ± 0.7	6.6 ± 1.6*	11.49	**	1.11	NS
Emotional excitation	− 0.3 ± 0.2	2.3 ± 0.5**	− 0.3 ± 0.3	4.7 ± 1.0***##	53.5	***	4.77	*
Inactivity	2.6 ± 0.7	9.0 ± 1.1**	1.3 ± 1.1	10.6 ± 2.7***	30.82	***	1.05	NS
Extroversion	− 0.5 ± 0.3	− 0.1 ± 0.6	0.1 ± 0.5	1.5 ± 0.7	2.67	NS	0.77	NS
Introversion	0.4 ± 0.1	4.1 ± 0.6***	0.5 ± 0.4	4.3 ± 0.8***	51.92	***	0.01	NS
Fear	− 0.1 ± 0.1	0.9 ± 0.3	− 0.4 ± 0.3	1.3 ± 1.0	9.51	**	0.72	NS
Dreaminess	0.2 ± 0.3	6.9 ± 0.7***	0.8 ± 0.5	7.9 ± 0.6***	160.2	***	0.11	NS
Vital signs								
Systolic blood pressure (mm Hg)	129 ± 2.0	142 ± 2.1***	133 ± 3.8	148 ± 2.9***	63.8	***	0.13	NS
Diastolic blood pressure (mm Hg)	76.9 ± 1.5	85.7 ± 1.7***	78.2 ± 2.0	87.6 ± 1.9***	68.8	***	0.08	NS
Heart rate (beats/min)	70.6 ± 1.8	79.1 ± 2.7**	72.8 ± 2.6	86.9 ± 4.29***	33.7	***	2.05	NS
Body temperature (Δ°C)	0.5 ± 0.1	0.8 ± 0.1**	0.3 ± 0.1	0.7 ± 0.1**	23.74	***	0.22	NS
Pupil size (mm)	6.1 ± 0.2	6.9 ± 0.1***	6.5 ± 0.2	7.2 ± 0.1***	61.08	***	0.81	NS
Pupil size after light (mm)	4.3 ± 0.2	5.2 ± 0.2***	4.6 ± 0.2	5.6 ± 0.2***	89.61	***	0.02	NS
List of complaints (Δ LC total score)								
Acute adverse effects (0–10 h)	0.5 ± 0.3	9.8 ± 1.8***	0.1 ± 0.6	10.4 ± 3.0***	38.37	***	0	NS
Subacute adverse effects (10–24 h)	− 0.2 ± 0.3	0.4 ± 0.2	− 0.4 ± 0.4	3.7 ± 1.4**	12.06	**	6.76	*
Subacute adverse effects (24–72 h)	− 0.5 ± 0.3	− 0.1 ± 0.2	− 0.8 ± 0.4	0.6 ± 0.9	6.03	*	1.83	NS

Values are mean ± SEM of the peak or peak changes (Δ) from baseline in 40 subjects. Sixteen subjects participated in the high dose study (200 µg) and 24 subjects in the moderate dose study (100 µg).

*for $p < 0.05$, **for $p < 0.01$, ***for $p < 0.001$ compared with placebo. # for $p < 0.05$, ## for $p < 0.01$, ### for $p < 0.001$ compared with LSD 100 µg.

psychedelic peak effects. These acute subjective effects of LSD and its effects on the emotion processing and behavioral tests in the present study are very similar to those of the prototypic empathogen MDMA. However, LSD induced higher AMRS intro- than extroversion while MDMA produced more extro- than introversion (Hysek *et al*, 2014a). Importantly, the subjective feelings of 'happiness', 'trust', 'closeness to others', and 'desire to be with others' at the high dose of LSD were maintained up to 6–12 h, and the effects of LSD on emotion processing and prosociality were

also observed late in time at 6–8 h after LSD administration and after the peak response when a 'plateau phase' was reached. At that time, the subjects were also less overwhelmed by initially strong and mostly novel psychedelic experiences, which may open a window for psychotherapeutic interventions. The emotional effects during the later phase of the acute LSD response (6–10 h) are likely beneficial to acutely facilitating the therapeutic alliance. Future research should address the relative contributions of the psychedelic peak experience vs empathogenic

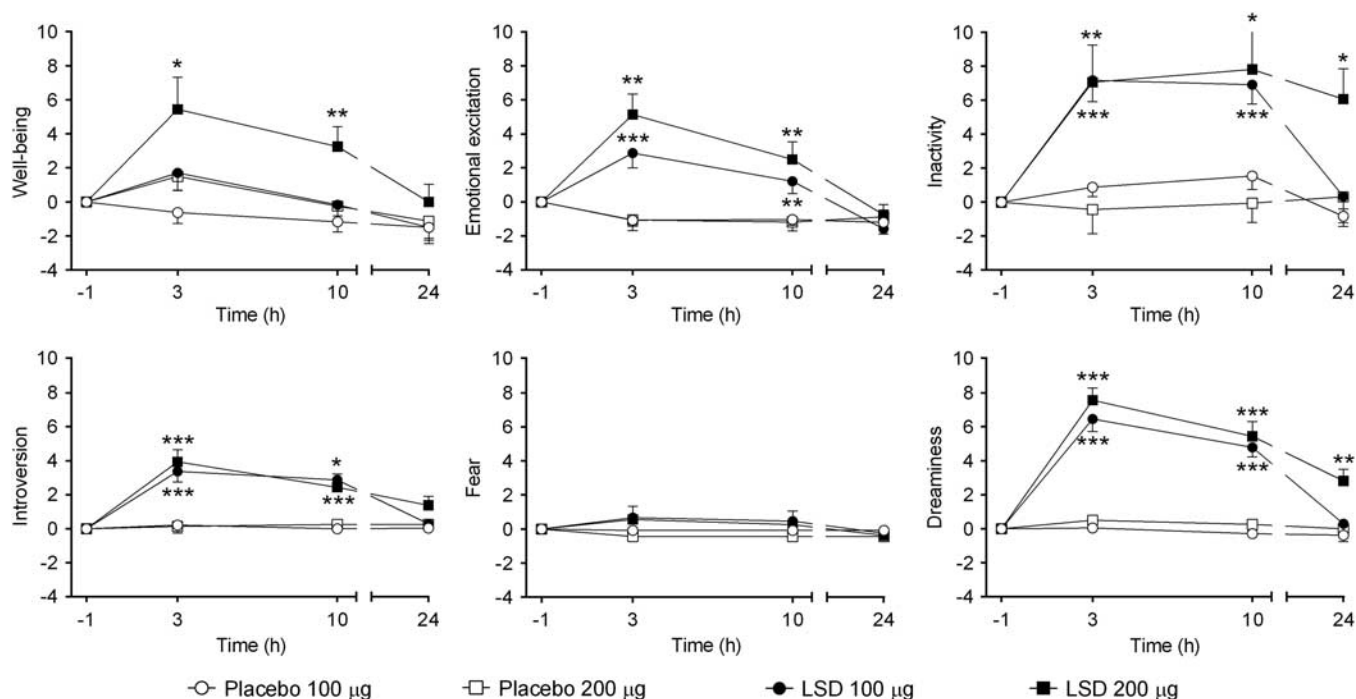


Figure 4 Subjective effects on the Adjective Mood Rating Scale. Lysergic acid diethylamide (LSD) or placebo was administered at $t=0$. The data are expressed as mean \pm SEM changes from baseline (-1 h) in 24 and 16 subjects in the 100 and 200 μg LSD dose groups, respectively. Emotion recognition (Face Emotion Recognition Task), empathy (Multifaceted Empathy Test), and social value orientation (SVO) tests were conducted 5–6 and 7–8 h after the administration of the 100 and 200 μg LSD dose, respectively. The corresponding maximal effects and statistics are shown in Table 1. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with placebo (T -tests).

emotional effects of LSD to its potential therapeutic effects. Additionally, it seems that only the higher 200 μg dose of LSD produced robust empathogenic effects. Furthermore, the relevance of deficits in cognitive empathy for the therapeutic process is unclear.

The present study also showed that LSD was well tolerated in a controlled setting in healthy subjects. Adverse effects of LSD mainly included acute dizziness, headache, and fatigue/exhaustion lasting up to 72 h. Both doses of LSD produced comparable moderate sympathomimetic effects including elevated blood pressure, heart rate, body temperature, and mydriasis.

The present study used two doses of LSD within a clinically relevant dose range. In fact, the higher dose was identical to both the amount and pharmaceutical formulation that were used in a clinical study in patients with anxiety (Gasser *et al*, 2014) and continue to be used in patients in Switzerland. Additionally, LSD was administered to subjects across a relatively wide age range (25–60 years). Importantly, the subjects typically had no or very limited hallucinogen experience, which is possibly similar to cases in which LSD is used therapeutically in patients. In contrast, other contemporary studies used lower doses of LSD in subjects with extensive prior substance use (Carhart-Harris *et al*, 2016, 2015). However, in the present study, previous hallucinogen use (1–3 times including LSD in six subjects) did not alter the responses to LSD.

In the present study, the tests were performed approximately 3 h after the peak effects (Dolder *et al*, 2015b; Schmid *et al*, 2015). At the time of the peak response of LSD, test administration would not have been feasible because of the

strong alterations in wake consciousness and impairments in concentration (Schmid *et al*, 2015). The participants needed to adjust to the altered state of consciousness; therefore, testing occurred after a 'plateau phase' was reached. Nevertheless, at the time of testing, the subjective effects and plasma concentrations of LSD were still at approximately 50% of the peak responses and clearly effective in producing typical LSD effects, providing a good time interval for conducting the neurocognitive tasks (Carhart-Harris *et al*, 2016; Schmid *et al*, 2015). Additionally, the tests were performed later after the high dose than after the low dose of LSD. However, at the times of testing, plasma LSD concentrations were twice as high after the 200 μg dose compared with the 100 μg dose, and generating a dose/concentration–response effect was possible.

The study has limitations. First, the dose effects of LSD were studied in different participants and not within-subject. Second, we assessed only emotion recognition and no other measures such as face muscle responses to emotions (Wardle *et al*, 2014) and the stimuli were artificial (pictures) rather than real people. With regard to the use of LSD in psychotherapy, we only assessed 'empathic concern for others' but not whether the participants 'felt cared for or understood by someone else' (Wardle and de Wit, 2014). It is possible that LSD affected attention and motivation and thereby task performance. Thus, it will be important to replicate and expand our findings using additional emotion recognition tests (Wardle and de Wit, 2014), tests of responses to emotions (Wardle and de Wit, 2014; Wardle *et al*, 2014), and other measures of social interaction (Frye *et al*, 2014).

In conclusion, LSD impaired emotion recognition of negative emotions and enhanced emotional empathy, particularly for positive emotional situations, and had subjective and behaviorally tested prosocial effects. These effects of LSD in healthy participants likely have translational relevance to LSD-assisted psychotherapy in patients and can be expected to reduce the perception of negative emotions and facilitate the therapeutic alliance.

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REFERENCES

- Bedi G, Hyman D, de Wit H (2010). Is ecstasy an 'empathogen'? Effects of \pm 3,4-methylenedioxymethamphetamine on prosocial feelings and identification of emotional states in others. *Biol Psychiatry* **68**: 1134–1140.
- Bogenschutz MP, Forcehimes AA, Pommy JA, Wilcox CE, Barbosa PC, Strassman RJ (2015). Psilocybin-assisted treatment for alcohol dependence: a proof-of-concept study. *J Psychopharmacol* **29**: 289–299.
- Carhart-Harris RL, Kaelen M, Bolstridge M, Williams TM, Williams LT, Underwood R et al (2016). The paradoxical psychological effects of lysergic acid diethylamide (LSD). *Psychol Med* **46**: 1379–1390.
- Carhart-Harris RL, Kaelen M, Whalley MG, Bolstridge M, Feilding A, Nutt DJ (2015). LSD enhances suggestibility in healthy volunteers. *Psychopharmacology* **232**: 785–794.
- Dolder PC, Liechti ME, Rentsch KM (2015a). Development and validation of a rapid turboflow LC-MS/MS method for the quantification of LSD and 2-oxo-3-hydroxy LSD in serum and urine samples of emergency toxicological cases. *Anal Bioanal Chem* **407**: 1577–1584.
- Dolder PC, Schmid Y, Haschke M, Rentsch KM, Liechti ME (2015b). Pharmacokinetics and concentration-effect relationship of oral LSD in humans. *Int J Neuropsychopharmacol* (doi:10.1093/ijnp/pyv072).
- Dziobek I, Rogers K, Fleck S, Bahnemann M, Heekeren HR, Wolf OT et al (2008). Dissociation of cognitive and emotional empathy in adults with Asperger syndrome using the Multifaceted Empathy Test (MET). *J Autism Dev Disord* **38**: 464–473.
- Frye CG, Wardle MC, Norman GJ, de Wit H (2014). MDMA decreases the effects of simulated social rejection. *Pharmacol Biochem Behav* **117**: 1–6.
- Gasser P, Holstein D, Michel Y, Doblin R, Yazar-Klosinski B, Passie T et al (2014). Safety and efficacy of lysergic acid diethylamide-assisted psychotherapy for anxiety associated with life-threatening diseases. *J Nerv Ment Dis* **202**: 513–520.
- Gasser P, Kirchner K, Passie T (2015). LSD-assisted psychotherapy for anxiety associated with a life-threatening disease: a qualitative study of acute and sustained subjective effects. *J Psychopharmacol* **29**: 57–68.
- Griffiths RR, Johnson MW, Richards WA, Richards BD, McCann U, Jesse R (2011). Psilocybin occasioned mystical-type experiences: immediate and persisting dose-related effects. *Psychopharmacology* **218**: 649–665.
- Griffiths RR, Richards WA, McCann U, Jesse R (2006). Psilocybin can occasion mystical-type experiences having substantial and sustained personal meaning and spiritual significance. *Psychopharmacology* **187**: 268–283 discussion 284–292.
- Grob CS, Danforth AL, Chopra GS, Hagerty M, McKay CR, Halberstadt AL et al (2011). Pilot study of psilocybin treatment for anxiety in patients with advanced-stage cancer. *Arch Gen Psychiatry* **68**: 71–78.
- Harmer CJ, Shelley NC, Cowen PJ, Goodwin GM (2004). Increased positive versus negative affective perception and memory in healthy volunteers following selective serotonin and norepinephrine reuptake inhibition. *Am J Psychiatry* **161**: 1256–1263.
- Hurlemann R, Patin A, Onur OA, Cohen MX, Baumgartner T, Metzler S et al (2010). Oxytocin enhances amygdala-dependent, socially reinforced learning and emotional empathy in humans. *J Neurosci* **30**: 4999–5007.
- Hysek CM, Domes G, Liechti ME (2012). MDMA enhances 'mind reading' of positive emotions and impairs 'mind reading' of negative emotions. *Psychopharmacology* **222**: 293–302.
- Hysek CM, Schmid Y, Simmler LD, Domes G, Heinrichs M, Eisenegger C et al (2014a). MDMA enhances emotional empathy and prosocial behavior. *Soc Cogn Affect Neurosci* **9**: 1645–1652.
- Hysek CM, Simmler LD, Schillinger N, Meyer N, Schmid Y, Donzelli M et al (2014b). Pharmacokinetic and pharmacodynamic effects of methylphenidate and MDMA administered alone and in combination. *Int J Neuropsychopharmacol* **17**: 371–381.
- Janke W, Debus G (1978). *Die Eigenschaftswörterliste*. Hogrefe: Göttingen.
- Johnson MW, Garcia-Romeu A, Cosimano MP, Griffiths RR (2014). Pilot study of the 5-HT_{2A}R agonist psilocybin in the treatment of tobacco addiction. *J Psychopharmacol* **28**: 983–992.
- Kirkpatrick MG, Gunderson EW, Perez AY, Haney M, Foltin RW, Hart CL (2012). A direct comparison of the behavioral and physiological effects of methamphetamine and 3,4-methylenedioxymethamphetamine (MDMA) in humans. *Psychopharmacology* **219**: 109–122.
- Kirkpatrick MG, Lee R, Wardle MC, Jacob S, de Wit H (2014). Effects of MDMA and intranasal oxytocin on social and emotional processing. *Neuropsychopharmacology* **39**: 1654–1663.
- Kometer M, Schmidt A, Bachmann R, Studerus E, Seifritz E, Vollenweider FX (2012). Psilocybin biases facial recognition, goal-directed behavior, and mood state toward positive relative to negative emotions through different serotonergic subreceptors. *Biol Psychiatry* **72**: 898–906.
- Kuypers KP, de la Torre R, Farre M, Yubero-Lahoz S, Dziobek I, Van den Bos W et al (2014). No evidence that MDMA-induced enhancement of emotional empathy is related to peripheral oxytocin levels or 5-HT_{1a} receptor activation. *PLoS One* **9**: e100719.
- MacLean KA, Johnson MW, Griffiths RR (2011). Mystical experiences occasioned by the hallucinogen psilocybin lead to increases in the personality domain of openness. *J Psychopharmacol* **25**: 1453–1461.
- Mithoefer MC, Wagner MT, Mithoefer AT, Jerome I, Doblin R (2010). The safety and efficacy of \pm 3,4-methylenedioxymethamphetamine-assisted psychotherapy in subjects with chronic, treatment-resistant posttraumatic stress disorder: the first randomized controlled pilot study. *J Psychopharmacol* **25**: 439–452.
- Mithoefer MC, Wagner MT, Mithoefer AT, Jerome L, Martin SF, Yazar-Klosinski B et al (2013). Durability of improvement in post-traumatic stress disorder symptoms and absence of harmful effects or drug dependency after 3,4-methylenedioxymethamphetamine-assisted psychotherapy: a prospective long-term follow-up study. *J Psychopharmacol* **27**: 28–39.
- Murphy RO, Ackermann KA, Handgraaf MJJ (2011). Measuring social value orientation. *Judgm Decis Mak* **6**: 771–781.

- Nichols DE (2016). Psychedelics. *Pharmacol Rev* **68**: 264–355.
- Oehen P, Traber R, Widmer V, Schnyder U (2013). A randomized, controlled pilot study of MDMA (\pm 3,4-methylenedioxymethamphetamine)-assisted psychotherapy for treatment of resistant, chronic post-traumatic stress disorder (PTSD). *J Psychopharmacol* **27**: 40–52.
- Passie T, Halpern JH, Stichtenoth DO, Emrich HM, Hintzen A (2008). The pharmacology of lysergic acid diethylamide: a review. *CNS Neurosci Ther* **14**: 295–314.
- Preller KH, Pokorny T, Krähenmann R, Dziobek I, Stämpfli P, Vollenweider FX (2015). The effect of 5-HT_{2A/1a} agonist treatment on social cognition, empathy, and social decision-making. *Eur Psychiatry* **30**(Suppl. 1): 22.
- Schmid Y, Enzler F, Gasser P, Grouzmann E, Preller KH, Vollenweider FX et al (2015). Acute effects of lysergic acid diethylamide in healthy subjects. *Biol Psychiatry* **78**(8): 544–553.
- Schmid Y, Hysek CM, Simmler LD, Crockett MJ, Quednow BB, Liechti ME (2014). Differential effects of MDMA and methylphenidate on social cognition. *J Psychopharmacol* **28**: 847–856.
- Strajhar P, Schmid Y, Liakoni E, Dolder PC, Rentsch KM, Kratschmar DV et al (2016). Acute effects of LSD on circulating steroid levels in healthy subjects. *J Neuroendocrinol* (doi:10.1111/jne.12374).
- Vollenweider FX, Vollenweider-Scherpenhuyzen MF, Babler A, Vogel H, Hell D (1998). Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action. *Neuroreport* **9**: 3897–3902.
- Wardle MC, de Wit H (2014). MDMA alters emotional processing and facilitates positive social interaction. *Psychopharmacology* **231**: 4219–4229.
- Wardle MC, Kirkpatrick MG, de Wit H (2014). 'Ecstasy' as a social drug: MDMA preferentially affects responses to emotional stimuli with social content. *Soc Cogn Affect Neurosci* **9**: 1076–1081.



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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (<http://www.nature.com/npp>)

Supplementary Information

Material and methods

Subjective effects

The Visual Analog Scales (VASs) were repeatedly used to assess subjective effects over time. The VASs included “any drug effect,” “good drug effect,” “drug high,” “bad drug effect,” “fear,” “happy,” “closeness to others,” “open,” “trust,” “I want to be hugged,” “I want to hug someone,” “I want to be alone,” and “I want to be with others” and have previously been used (Hysek *et al*, 2014a; Schmid *et al*, 2015). The VASs were presented as 100-mm horizontal lines (0-+100%) marked from “not at all” on the left and “extremely” on the right. The VASs for “happy,” “closeness to others,” “open,” “trust,” “I want to be hugged,” “I want to hug someone,” “I want to be alone,” and “I want to be with others” were bidirectional ($\pm 50\%$), marked from “not at all” on the left (-50), to “normal” in the middle (0), to “extremely” on the right (+50). The VASs were administered 1 h before and 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 16, and 24 h after drug administration.

The 60-item Likert-scale short version of the Adjective Mood Rating Scale (AMRS) (Janke and Debus, 1978) was administered 1 h before and 3, 10, and 24 h after placebo or LSD. The AMRS contains subscales for well-being, emotional excitation, activity, inactivity, extro- and introversion, fear, and dreaminess. The AMRS has previously been shown to be sensitive to the effects of psychostimulants, empathogens, and hallucinogens (Hasler *et al*, 2004; Hysek *et al*, 2014b; Schmid *et al*, 2015).

Vital signs, pupillary function, and adverse effects

Blood pressure, heart rate and body temperature were assessed repeatedly 1 h before and 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 24 h after drug administration. Blood pressure (systolic and diastolic) and heart rate were measured using an automatic oscillometric device (OMRON Healthcare Europe NA, Hoofddorp, Netherlands). The measurements were performed in duplicate at an interval of 1 min and after a resting time of at least 10 min. The averages were calculated for analysis. Core (tympanic) temperature was measured using an GENIUSTM 2 ear thermometer (Tyco Healthcare Group LP, Watertown, NY, USA).

Pupillometry was performed 1 h before and 1, 2.5, 4, 7, and 11 h after drug administration using an infrared pupillometer (PRL-200, NeuroOptics, Irvine, CA, USA) under standardized dark-light conditions as previously described (Hysek and Liechti, 2012). The dark-adapted maximal pupil diameter, minimal pupil diameter after a light stimulus, and constriction amplitude (difference between maximal and minimal pupil size) were recorded.

Adverse effects were assessed using the 66-item List of Complaints (Zerssen, 1976) before and 10, 24, and 72 h after drug administration for the 0-10, 10-24, and 24-72 h time intervals, respectively. Complaints are assessed as present or not present and there is no grading of the complaint. However, the scale yields a total adverse effects score, reliably measuring general discomfort.

References

- Hasler F, Grimberg U, Benz MA, Huber T, Vollenweider FX (2004). Acute psychological and physiological effects of psilocybin in healthy humans: a double-blind, placebo-controlled dose-effect study. *Psychopharmacology* 172: 145-156.
- Hysek CM, Liechti ME (2012). Effects of MDMA alone and after pretreatment with reboxetine, duloxetine, clonidine, carvedilol, and doxazosin on pupillary light reflex. *Psychopharmacology* 224: 363-376.
- Hysek CM, Schmid Y, Simmler LD, Domes G, Heinrichs M, Eisenegger C, et al (2014a). MDMA enhances emotional empathy and prosocial behavior. *Soc Cogn Affect Neurosci* 9: 1645-1652.
- Hysek CM, Simmler LD, Schillinger N, Meyer N, Schmid Y, Donzelli M, et al (2014b). Pharmacokinetic and pharmacodynamic effects of methylphenidate and MDMA administered alone and in combination. *Int J Neuropsychopharmacol* 17: 371-381.
- Janke W, Debus G (1978). Die Eigenschaftswörterliste. Hogrefe: Göttingen.
- Schmid Y, Enzler F, Gasser P, Grouzmann E, Preller KH, Vollenweider FX, et al (2015). Acute effects of lysergic acid diethylamide in healthy subjects. *Biol Psychiatry* 78: 544-553.
- Zerssen DV (1976). Die Beschwerden-Liste. Münchener Informationssystem Psychis, München.

Table S1. Life-time prevalence of substance use

Subject	MDMA	amphetamine	cocaine	LSD	psilocybin	methylphenidate	mescaline	THC
Study 1								
1	2	0	0	0	0	0	0	0
2	1	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	5-10
4	3	0	1	0	0	0	0	10-20
5	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0
7	5	1	1	1	0	0	0	0
8	1	0	0	0	0	2	0	0
9	0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0	5-10
11	0	0	0	0	0	0	0	0
12	5	0	0	0	0	0	0	5-10
13	0	0	0	0	0	0	0	0
14	0	0	0	0	0	0	0	5-10
15	0	0	0	0	0	0	0	10-20
16	0	0	0	0	1	0	0	5-10
17	0	0	0	1	0	0	0	0
18	0	0	0	1	1	0	0	0
19	2	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	2
21	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0
23	3	1	0	0	0	1	0	0
24	0	0	0	0	0	0	0	0
Study 2								
1	4	0	0	0	0	2	0	1
2	2	0	0	0	1	0	1	5-10
3	4	0	0	1	0	2	0	10-20
4	0	0	0	0	3	0	0	5-10
5	2	0	0	0	0	0	0	50-100
6	0	0	0	0	0	0	0	100-200
7	3	0	2	2	0	0	0	100-200
8	2	0	0	0	0	0	0	0
9	1	0	0	0	2	0	0	5-10
10	0	0	0	0	0	0	0	100-200
11	0	0	0	0	0	0	0	20-50
12	2	1	2	0	1	0	0	50-100
13	8	0	0	2	0	0	0	50-100
14	0	0	0	0	0	1	0	2
15	3	1	1	0	0	1	0	1
16	0	0	0	0	0	0	0	5-10

Values are times used in life

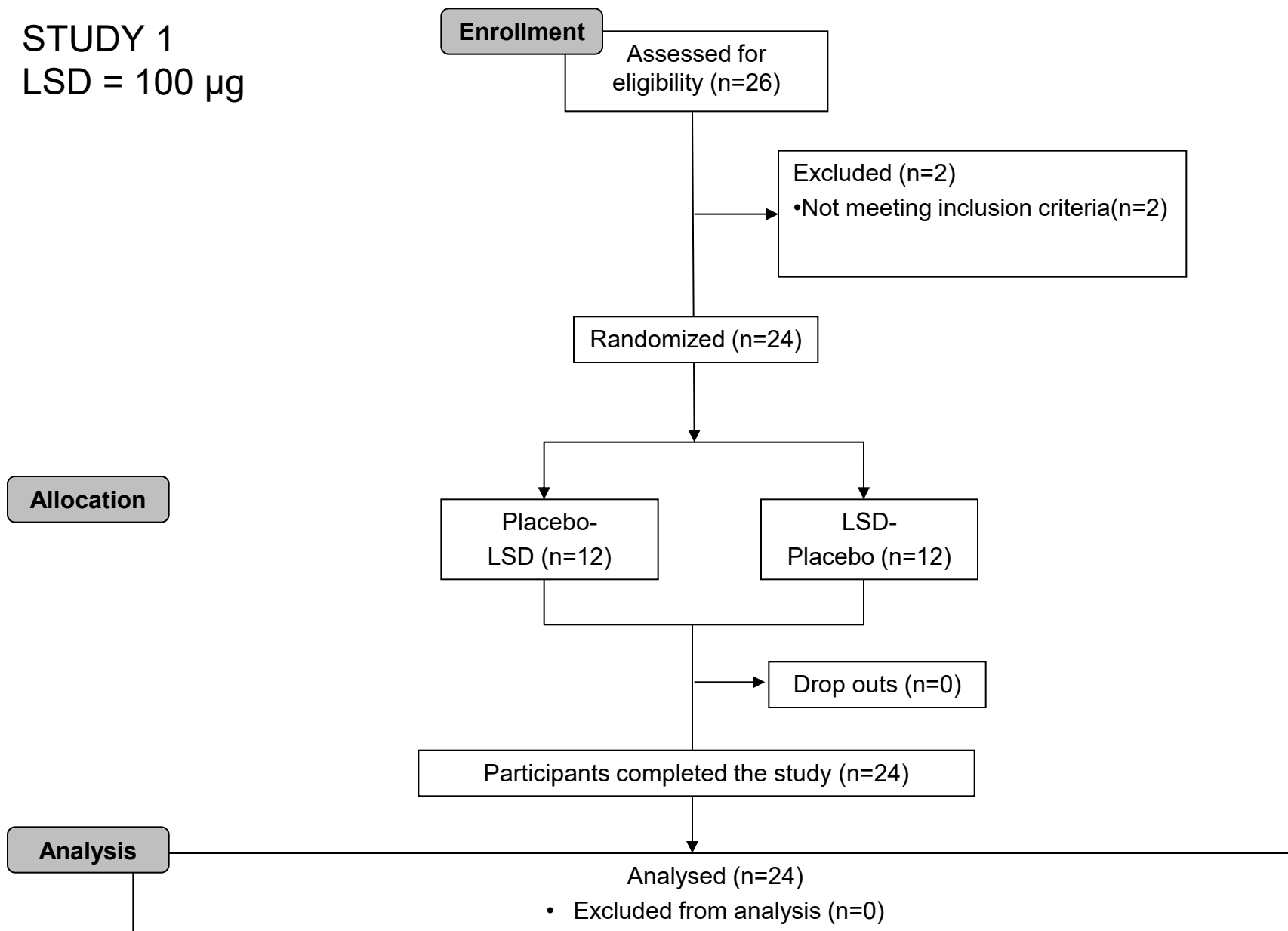
Table S2. Percent of acute and sub-acute adverse drug effects up to 72 hours.

Study 1	Placebo 100 µg				LSD 100 µg			
	0h	0-10h	10-24h	24-72h	0h	0-10h	10-24h	24-72h
Difficulty concentrating	0%	0%	0%	0%	0%	54%	0%	0%
Headache	0%	29%	13%	0%	4%	58%	17%	21%
Exhaustion	4%	13%	4%	0%	4%	25%	4%	4%
Dizziness	0%	0%	0%	0%	0%	25%	0%	0%
Lack of appetite	0%	0%	0%	0%	0%	46%	0%	4%
Dry mouth	4%	4%	0%	0%	0%	29%	0%	0%
Imbalance	0%	0%	0%	0%	0%	42%	0%	0%
Nausea	0%	0%	0%	0%	0%	21%	0%	0%
Fatigue	29%	29%	13%	4%	17%	58%	29%	33%

Study 2	Placebo 200 µg				LSD 200 µg			
	0h	0-10h	10-24h	24-72h	0h	0-10h	10-24h	24-72h
Difficulty concentrating	0%	4%	4%	0%	0%	42%	13%	8%
Headache	4%	13%	8%	0%	8%	38%	33%	8%
Exhaustion	4%	4%	4%	0%	4%	29%	42%	13%
Dizziness	4%	0%	0%	0%	0%	29%	8%	0%
Lack of appetite	0%	0%	0%	0%	4%	21%	13%	0%
Dry mouth	0%	0%	0%	0%	0%	21%	13%	4%
Imbalance	0%	0%	0%	0%	0%	21%	4%	0%
Nausea	4%	4%	4%	0%	8%	17%	0%	0%
Fatigue	25%	17%	17%	0%	38%	38%	46%	17%

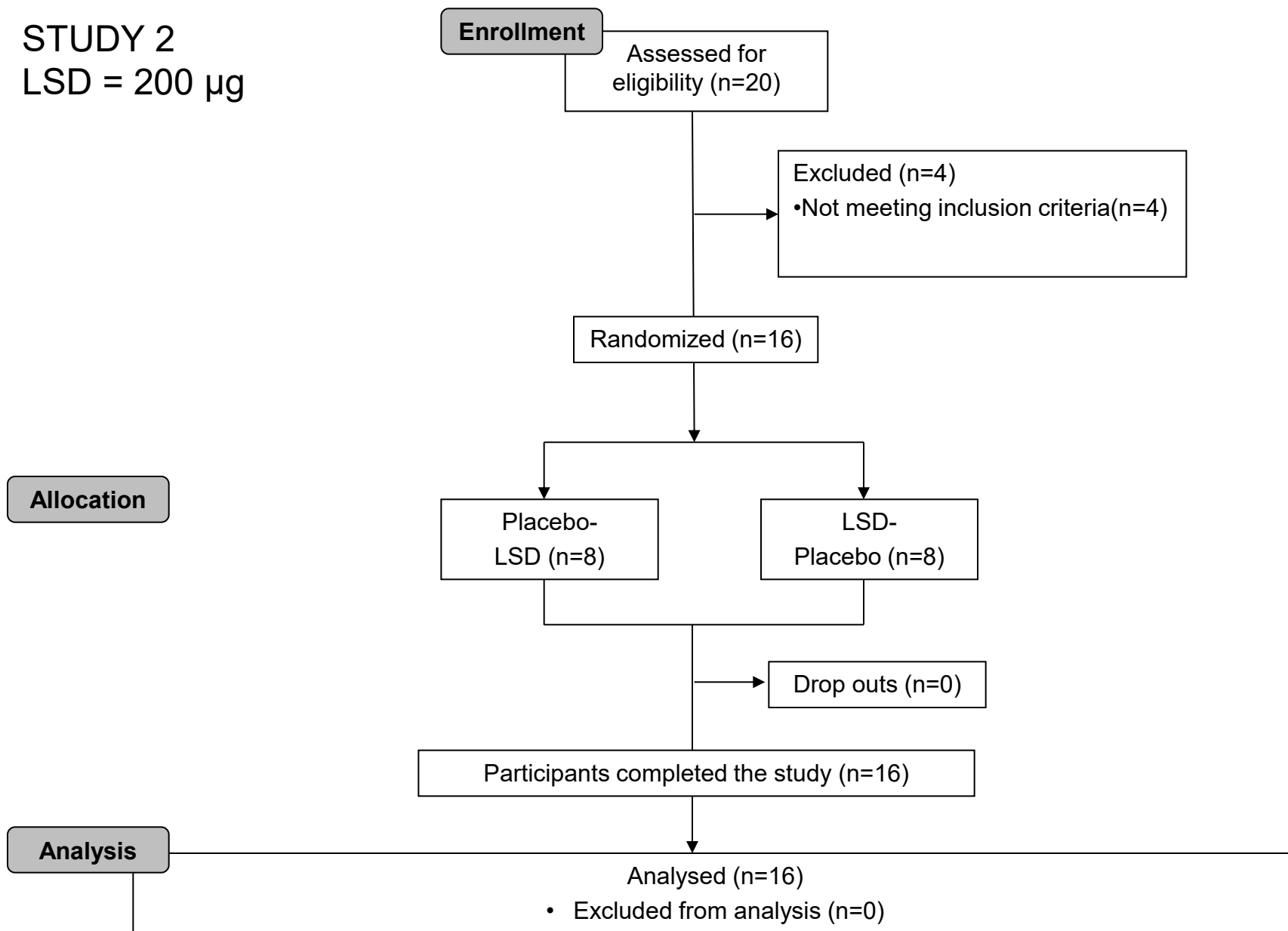
Data percent in 16 and 24 subjects for the high and low dose group, respectively.

STUDY 1
LSD = 100 µg



STUDY 2

LSD = 200 µg



3.5 Publication 5

Acute effects of LSD on amygdala activity during processing of fearful stimuli in healthy subjects

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ORIGINAL ARTICLE

Acute effects of LSD on amygdala activity during processing of fearful stimuli in healthy subjects

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Lysergic acid diethylamide (LSD) induces profound changes in various mental domains, including perception, self-awareness and emotions. We used functional magnetic resonance imaging (fMRI) to investigate the acute effects of LSD on the neural substrate of emotional processing in humans. Using a double-blind, randomised, cross-over study design, placebo or 100 µg LSD were orally administered to 20 healthy subjects before the fMRI scan, taking into account the subjective and pharmacological peak effects of LSD. The plasma levels of LSD were determined immediately before and after the scan. The study (including the *a priori*-defined study end point) was registered at ClinicalTrials.gov before study start (NCT02308969). The administration of LSD reduced reactivity of the left amygdala and the right medial prefrontal cortex relative to placebo during the presentation of fearful faces ($P < 0.05$, family-wise error). Notably, there was a significant negative correlation between LSD-induced amygdala response to fearful stimuli and the LSD-induced subjective drug effects ($P < 0.05$). These data suggest that acute administration of LSD modulates the engagement of brain regions that mediate emotional processing.

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INTRODUCTION

Lysergic acid diethylamide (LSD), a potent psychoactive substance,¹ induces profound changes in various mental domains, including perception, self-awareness and emotions.^{2,3} As with the other psychedelics (for example, psilocybin and mescaline), these effects are mainly mediated through agonism at the serotonin 5-HT_{2A} receptor.^{1,4} Currently, there are renewed efforts to use substances like LSD and psilocybin in basic research and clinical practice.^{2,3,5,6,7} Psilocybin has been studied as a treatment option for addiction, depression and for anxiety in patients with advanced stage cancer.^{8–11} LSD has been shown to reduce anxiety in patients with life-threatening diseases.¹² With the investigation of its basic pharmacological and psychological effects, there is also rising interest in the neuronal correlates of the LSD-induced altered state of consciousness. Although several modern studies on psilocybin have been conducted, recent data on LSD in humans are still very limited.¹

Functional neuroimaging provides a sensitive means of examining how LSD acts on the brain. No data investigating LSD effects on emotion processing have yet been published. The aim of the present study was therefore to investigate these acute effects of LSD using functional magnetic resonance imaging (fMRI). Using a double-blind, randomised, cross-over study design, placebo or 100 µg LSD were orally administered to 20 physically and mentally healthy participants 2.5 h before the fMRI scan, taking into account the subjective and pharmacological peak effects of LSD.^{2,6} Subjects only had minimal lifetime exposure to illicit drugs; notably, only two subjects had had prior experience with a psychedelic, both on one occasion only. During the fMRI scan, human fearful and neutral faces of a well-validated paradigm were presented. To test our hypothesis that there were differences

between placebo and LSD during processing of emotional stimuli, trials for fearful faces were contrasted against trials for neutral faces. We thereby focused on the amygdala as one central part of neural emotion processing, in particular, of anxiety^{13,14} and additionally included two other regions (the fusiform gyrus and the medial frontal gyrus) known to be responsive to fearful faces.¹⁴ Differences between placebo and LSD conditions were evaluated by second-level paired *t*-test analysis. In addition, the amygdala response to fearful faces after LSD was correlated with the subjective drug effect, as assessed by a visual analogue scale directly before the scan. The primary and *a priori*-defined study hypothesis was that LSD would decrease the amygdala response to fearful stimuli and that this decrease would be associated with the subjective psychedelic effects.

MATERIALS AND METHODS

We used a randomised, placebo-controlled, double-blind, cross-over design. Each participant completed two study sessions, with a washout period of at least 7 days between the sessions. The study was approved by the Ethics Committee for Northwest/Central Switzerland (EKNZ) and by the Federal Office of Public Health. Written informed consent was obtained from all the participants. The study (including the *a priori*-defined study end point) was registered at clinicaltrials.gov before study start (NCT02308969).

Subjects

The subjects were recruited by advertisement and word of mouth. The sample size was determined by power analysis based on previous data.^{15,16} The exclusion criteria were age < 25 or > 65 years, pregnancy (as determined by urine test), nursing, hypertension (> 140/90 mm Hg) or hypotension (systolic blood pressure < 85 mm Hg), cardiac or neurological disorders, use of any regular medication, as determined by medical history

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and general medical examination including electrocardiography, blood chemistry and haematology, use of illicit drugs (except cannabis) >10 times or any time within the previous 2 months (as assessed by the history and urine test for benzodiazepines, cocaine, amphetamines, methadone, opiates and barbiturates), smoking of >10 cigarettes per day, history of drug dependence, personal or first-degree relative with a history of seizures, personal or first-degree relative with an axis I major psychiatric disorder (as determined by general medical history and a semi-structured interview for Diagnostic and Statistical Manual of Mental Disorders, fourth edition). The subjects provided written informed consent and received monetary compensation for their participation.

Study procedure

The study included a screening visit, two 25 h test sessions and an end of study visit. The experimental sessions took place in a quiet room in the University Hospital of Basel, Switzerland. The study dates were between December 2014 (first subject screened) and September 2015 (last end of study session). The participants were monitored for adverse reactions and events during the study dates and at the end of study visit. All the adverse events were recorded. The participants were instructed to abstain from any illicit drugs during the whole study period and, additionally, to abstain from caffeine, chocolate and alcohol for at least 8 h before the sessions. The urine drug tests (for tetrahydrocannabinol, benzodiazepines, cocaine, amphetamines, methadone, opiates and barbiturates) were taken randomly on one of the two sessions. In women, pregnancy tests were performed before every session. Except for tetrahydrocannabinol, which can be detected for several weeks, detection of any drug of abuse resulted in study exclusion. A light standardised breakfast was served at both the sessions. Placebo and LSD were administered orally, 2.5 h before the MRI scan at 0900 h, taking into account the subjective and pharmacological peak effects of LSD.^{2,6}

Drugs and randomization

Gelatin capsules containing 100 µg D-lysergic acid diethylamide hydrate (Lipomed, Arlesheim, Switzerland) and identical capsules containing mannitol were prepared. Each subject received either placebo or LSD on two study sessions in a counterbalanced manner. Only the person dispensing the substance (who was not further involved in conducting the study) was aware of the treatment assignment. Subjects and study personnel were blind to the treatment order.

Image acquisition

Scanning was conducted on a 3 Tesla MRI system (Magnetom Prisma, Siemens Healthcare, Erlangen, Germany), using a 20-channel phased array radio frequency head coil. Functional MRI acquisition was based on an interleaved T2*-weighted echo planar imaging sequence, with 39 axial slices with a slice thickness of 3 mm, a 0.5 mm inter-slice gap, a field-of-view of 228 × 228 cm² and an in-plane image matrix size of 76 × 76—resulting in 3 × 3 × 3 mm³ resolution. The corresponding repetition time was 2.5 s, echo time 30 ms and bandwidth = 2350 Hz per pixel. In total, 152 volumes were acquired (including three dummy scan volumes to ensure signal stabilization).

Subjective effect measurements

The visual analogue scale 'Any subjective drug effects' was used to assess the overall subjective response to LSD before the scan. The visual analogue scale was presented as a 100 mm horizontal line (0–100%) marked 'not at all' on the left and 'extreme' on the right. The scale was rated by the volunteers 2 h after the administration of LSD or placebo.

Plasma levels

The blood was collected into lithium heparin tubes 2 and 3 h after the administration of LSD and placebo, respectively. The blood samples were immediately centrifuged and rapidly stored at –20 °C until analysis. LSD concentrations in plasma were determined using a validated liquid chromatography-tandem mass spectrometry method.⁶

fMRI paradigm

During the fMRI acquisition, the study subjects participated in a 6 min experiment based on event-related design implemented with E-Prime 2.0 (Psychology Software Tools, Pittsburgh, PA, USA). During the task,

participants were presented with 10 different facial identities (pictures of human faces from the Ekman & Friesen series of Pictures of Facial Affect), each expressing 50 or 100% intensities of fear or a neutral expression. There were thus 30 different facial stimuli in total. Each face was shown twice for 2 s, resulting in a total of 60 stimuli during the paradigm. The order of facial identities and expression type was pseudo-randomised to prevent successive presentation of the same identity or facial expression type. The length of the interstimulus interval, during which subjects viewed a fixation cross, was varied from 3 to 8 s according to a Poisson distribution, with an average interval of 5.9 s. To ensure maximal attention to the presented faces, subjects were requested to decide on the gender of face stimuli by pressing a left or a right button. Accuracy and reaction times were monitored and recorded.

Data analysis

The data analysis was performed using SPM12 (<http://www.fil.ion.ucl.ac.uk/spm/>). All the volumes were slice time corrected, realigned to the first volume, co-registered to the pre-processed T₁-weighted structural volume, normalized into a standard stereotactic space (Montreal Neurological Institute, MNI) and smoothed with a 6 mm full width at half maximum Gaussian kernel. The dummy scans were excluded from any further processing and the remaining volumes were quality checked for severe head motion and image artefacts. The subjects with head motion of >2 mm translation or >2° rotation were excluded. During model specification, the onset times for each trial of neutral, 50% and 100% fearful faces were convolved with a canonical haemodynamic response function. The serial correlations were removed with a first-order autoregressive model and a high-pass filter (128 s) was applied to remove low frequency noise. The six motion parameters for translation and rotation were entered as nuisance covariates. In addition, time and dispersion derivatives were included in the individual design matrix during the first-level analysis. Each trial for 50 and 100% fearful faces was then contrasted against neutral faces, and then produced a subject-specific contrast image propagated to the second-level analysis. One-sample *t*-tests were used to assess the activity induced by the main effect task over all included subjects. The threshold over the whole brain was set at *P* = 0.05, corrected for multiple comparisons (family-wise error, FWE). Differences between the LSD and placebo treatment were evaluated by a second-level paired *t*-test. Whole-brain threshold was set at *P* = 0.001, uncorrected for multiple comparisons, with an extent threshold of *k* = 10 voxels. We restricted our analysis to three meta-analytically identified¹⁴ regions of interest, namely the amygdala, the fusiform gyrus and the medial frontal gyrus. Those regions were specifically described to be involved in the processing of fearful faces compared with neutral faces.¹⁴ Based on the Harvard-Oxford Atlas for cortical and subcortical structures, a mask comprising those regions was created. Small volume correction was used for clusters observed within this hypothesized region of interest. The statistical threshold was adjusted to provide a FWE of *P* < 0.05, corrected for small volumes. The small volume correction was performed in the global maximum, with a sphere of 5 mm, in accordance with previous fMRI studies on amygdala activity.^{17,18}

The correlation with the subjective effect of LSD in the visual analogue scale was performed using the extracted beta values of the amygdala cluster under the LSD condition. We thereby used the '100% fearful versus neutral contrast' to obtain the distinct effect of the fearful stimuli. The calculations were performed using SPSS version 23.00 (IBM, Zurich, Switzerland).

RESULTS

We included data sets from 20 healthy subjects—9 men, 11 women; mean age 32 ± 10.2 years; range: 25–58 years, all right-handed and all but one with an academic background, originally with 24 study participants. The data sets from four subjects were excluded because of artefacts due to head movements. The lifetime drug use of the 20 included subjects is shown in Table 1. None of the participants tested positive for any drug (including tetrahydrocannabinol) in the screening or test session. No serious adverse reactions or events occurred during the whole period of the study in any of the participants. The plasma levels of LSD were determined immediately before and after the scan and were 1.3 ± 0.6 ng ml^{–1} (mean ± s.d.) and 1.1 ± 0.5 ng ml^{–1} (mean ± s.d.), respectively.

Task performance

The differences between the LSD and placebo conditions in task performance were assessed using paired *t*-tests. The mean subject

Table 1. Cumulative lifetime use of legal and illicit drugs of the included subjects

<i>Nicotine</i>		<i>Stimulants</i>	
No. of subjects with regular use	6/20	No. of subjects who have ever used	4/20
Cigarettes per day (mean/s.d./range)	1.40/4.03/0–10	Lifetime occasions (mean/s.d./range)	0.35/0.5/0–2
<i>Caffeine</i>		<i>Sedatives</i>	
No. of subjects with regular use	20/20	No. of subjects who have ever used	0/20
Units per day (mean/s.d./range)	3.05/1.96/1–8	Lifetime occasions (mean/s.d./range)	0/0/0
<i>Alcohol</i>		<i>Psychedelics</i>	
No. of subjects with regular use	20/20	No. of subjects who have ever used	2/20
Units per week (mean/s.d./range)	4.50/2.89/1–10	Lifetime occasions (mean/s.d./range)	0.10/0/0–1
<i>Cannabis</i>		<i>Opioids</i>	
No. of subjects who have ever used	15/20	No. of subjects who have ever used	1/20
Lifetime occasions (mean/s.d./range)	7.85/13.39/1–50	Lifetime occasions (mean/s.d./range)	0.05/0/0–1
<i>MDMA</i>		<i>Others</i>	
No. of subjects who have ever used	6/20	No. subjects who have ever used	0/20
Lifetime occasions (mean/s.d./range)	0.60/0.89/0–3	Lifetime occasions (mean/s.d./range)	0/0/0

Abbreviation: MDMA, 3,4-methylenedioxymethamphetamine.

response times did not differ significantly between the two conditions (LSD: 964 ± 128 ms (mean \pm s.d.); placebo: 910 ± 289 ms (mean \pm s.d.); $t_{21} = 2.0$, $P = 0.06$). Furthermore, no significant differences were found between the conditions in correctness of response (LSD: $93.1 \pm 10.8\%$ (mean \pm s.d.); placebo: $97.3 \pm 3.3\%$ (mean \pm s.d.); $t_{21} = -1.8$, $P = 0.08$) or absence of button presses (LSD: $4.5 \pm 9.3\%$ (mean \pm s.d.); placebo: $1.3 \pm 1.8\%$ (mean \pm s.d.); $t_{21} = 1.5$, $P = 0.16$).

Effect of task

With both treatments ($n = 40$), viewing neutral faces versus baseline was associated with bilateral activation in a network comprising the cerebellum, fusiform gyrus, occipital gyrus and the middle cingulate gyrus and lateral activation in the left frontal and lingual gyrus (FWE-corrected at $P < 0.05$).

Viewing 100 and 50% fearful faces versus baseline was associated with bilateral activation in the cerebellum, fusiform gyrus, occipital gyrus, middle superior parietal lobule and lateral activation in the left cingulate and frontal gyrus (FWE-corrected at $P < 0.05$). Under the placebo condition, presentation of fearful faces induced a significant (small volume correction, $P < 0.05$ FWE cluster level) activation of the left amygdala (MNI_{\max} $x = -20$, $y = -12$, $z = -12$; cluster size 22; Z -score 3.59) compared with presentation of neutral faces.

Effect of LSD on neural response to fearful versus neutral faces

Compared with placebo, administration of LSD reduced neural response to fearful versus neutral faces in the left and right amygdala and the medial frontal gyrus ($P < 0.001$, $k = 10$; see Figure 1). No increased activity was observed. After correction for multiple comparisons (small volume correction, $P < 0.05$ FWE), significantly reduced activity was observed in the left amygdala (MNI_{\max} $x = -15$, $y = 9$, $z = -14$; cluster size 24; Z -score 3.12) and the right medial frontal gyrus (MNI_{\max} $x = 15$, $y = 42$, $z = 16$; cluster size 12; Z -score 3.78). In addition, there was a significant negative correlation between amygdala blood oxygen-level dependent response to fearful stimuli under the LSD condition and the LSD-induced subjective drug effects ($r = -0.46$, $P < 0.05$; see Figure 2).

DISCUSSION

In summary, the present study used fMRI for we believe the first time to investigate the effects of LSD on the neural substrate of emotional processing. We found that LSD decreased amygdala

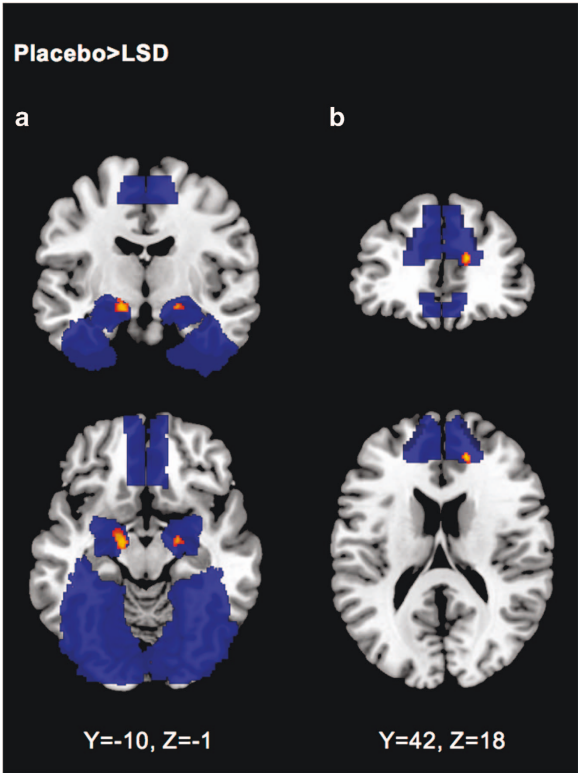


Figure 1. Neural response to fearful versus neutral faces after placebo compared with LSD treatment. LSD decreased reactivity (shown in red-yellow) to fearful faces in the amygdala (a) and the right medial frontal gyrus (b). Regions of interest (amygdala, fusiform gyrus, medial frontal gyrus) are shown in blue. Threshold $P < 0.001$, $k = 10$. Right is right side of the brain. LSD, lysergic acid diethylamide.

reactivity to fearful stimuli in healthy subjects. In addition, amygdala deactivation by LSD was associated with its acute subjective psychedelic effects. We administered $100 \mu\text{g}$ LSD, a representative dose that produces typical and robust psychedelic effects.¹⁹ In addition, subjects had only had a minimal exposure to recreational drugs and were mostly psychedelic-naïve, as is probably the case in patients receiving LSD-assisted

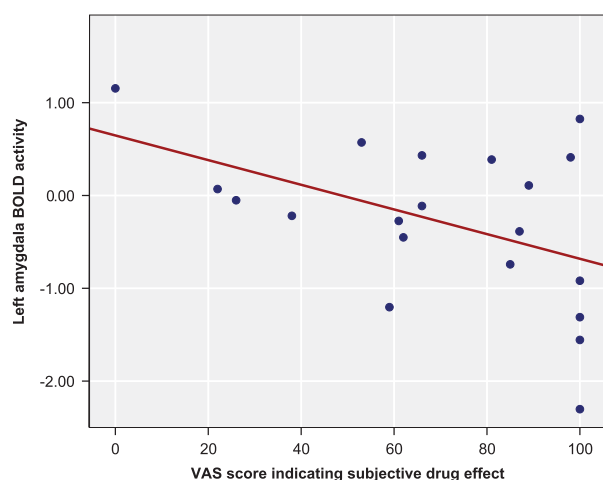


Figure 2. Relation between left amygdala BOLD activity during presentation of fearful faces under the LSD condition and visual analogue scale (VAS) for 'any subjective drug effects' of LSD ($r = -0.46$, $P < 0.05$). BOLD, blood oxygen-level dependent; LSD, lysergic acid diethylamide.

psychotherapy.¹² Our results are consistent with our previously reported findings in a facial emotion recognition task, showing that LSD-impaired recognition of fearful faces compared with placebo.²⁰ Our results are also in line with findings obtained after administration of psilocybin, where attenuated recognition of negative facial expressions^{21,22} and reduced amygdala blood oxygen-level dependent response to fearful faces²³ were reported. The psilocybin-induced attenuation of amygdala reactivity in response to negative stimuli has consistently been shown to be related to the psilocybin-induced increase in positive mood.²³

It could be argued that the decreased responsiveness of the amygdala under LSD was due to a drug-induced alteration in visual perception, resulting in the inability to differentiate between the presented facial expressions. However, our results in two doses of LSD (100 µg and 200 µg, respectively) indicated, that LSD specifically impaired recognition of fearful faces, while it did not significantly affect recognition of neutral, happy and angry faces.²⁰ Furthermore, subjects in the present study performed well in the gender differentiation task and our whole brain results showed activation in regions typically involved in processing of neutral and fearful faces, respectively.¹⁴

We observed a significant effect of LSD on the left amygdala. Several studies suggest, that the left amygdala might be particularly involved in processing of negative facial expressions.^{24,25} It has also been reported that the left amygdala shows lesser habituation to fearful stimuli compared with the right amygdala, which might make it more likely to detect the blood oxygen-level dependent changes in this area.^{26,27} However, lateralization of the amygdala response is still controversial discussed.^{28–30} The amygdala receives considerable serotonergic innervation from the raphe nuclei.^{31,32} LSD mainly acts as an agonist at the serotonin 5-HT_{2A} receptor,^{1,4} which is expressed in most parts of the amygdala.³³ Accordingly, this might provide a psychopharmacological basis for the observed effect of LSD on this structure. After administration of selective serotonin reuptake inhibitors in healthy subjects, a decreased amygdala activity in response to negative stimuli was reported.^{34,35} In depressed patients, amygdala hyperreactivity was resolved after treatment with a selective serotonin reuptake inhibitor.^{36,37} Those findings indicate that the serotonin system is involved in the modulation of the amygdala response to emotional stimuli.³⁸ We also observed decreased activity in the right medial prefrontal cortex (mPFC). The mPFC is anatomically connected to the amygdala³⁹ and

involved in emotional functions.⁴⁰ Within the mPFC–amygdala circuit, the more ventral parts of the mPFC have been implicated in inhibitory functions,⁴⁰ whereas the more dorsal parts are thought to be part of an 'aversive-amplification circuit'.⁴¹ This mechanism has been linked to negative affective bias in anxiety disorder.^{42,43} Consistent with our findings, serotonin depletion has been shown to increase mPFC activity and functional connectivity between the mPFC and the amygdala in response to fearful stimuli.⁴⁴

The use of psychedelics as an additive in psychotherapy has recently been rediscovered^{10,12,45} and our result is relevant for this field of research. Processing biases towards negative stimuli are a feature of several mental diseases, such as depression and social anxiety disorder, and are associated with increased reactivity of the amygdala.^{46,47} Resolving this processing bias might thus reflect one important and potentially therapeutically useful effect of psychedelic substances by, for example, facilitating the therapeutic alliance^{48,49} and reducing perception of negative emotions and social cognitive deficits. As we have recently reported, LSD also exhibits some 'empathogenic' effects (such as increased openness and trust),^{2,20} which are usually ascribed to substances like 3,4-methylenedioxymethamphetamine (MDMA). The attenuated amygdala reactivity observed in this study is in good accordance with those findings and possibly reflects a neural basis for such effects, which might also be therapeutically beneficial.^{49,50} However, and in contrast to substances like selective serotonin reuptake inhibitors, the positive long-term effects of psychedelics reported by recent studies^{8–12,15,51} outlast the acute pharmacological effects. It should be further investigated how psychological and biological factors, like neuroplasticity,⁵² contribute to these long-term effects.

Our study has several limitations. First, although the trial was formally double-blinded, assignment to placebo or LSD was unavoidably unblinded by the obvious psychedelic effects caused by the dose used. Second, we did not include in our analyses measures of negative affect. Third, we can only provide data about one moderate dose. Higher doses of psychedelics are possibly difficult to use with fMRI, because they are more likely to induce anxiety,⁴⁵ although the overall effects are still described as positive in the higher doses investigated.^{2,45} The observed anxiolytic effect probably also depends on personal and environmental factors and might thus be different in the mentally ill or in uncontrolled settings.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

- Nichols DE. Psychedelics. *Pharmacol Rev* 2016; **68**: 264–355.
- Schmid Y, Enzler F, Gasser P, Grouzmann E, Preller KH, Vollenweider FX et al. Acute effects of lysergic acid diethylamide in healthy subjects. *Biol Psychiatry* 2015; **78**: 544–553.
- Carhart-Harris RL, Kaelen M, Bolstridge M, Williams TM, Williams LT, Underwood R et al. The paradoxical psychological effects of lysergic acid diethylamide (LSD). *Psychol Med* 2016; **46**: 1–12.
- Vollenweider FX, Vollenweider-Scherpenhuyzen MF, Babler A, Vogel H, Hell D. Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action. *Neuroreport* 1998; **9**: 3897–3902.
- Carhart-Harris RL, Kaelen M, Whalley MG, Bolstridge M, Feilding A, Nutt DJ. LSD enhances suggestibility in healthy volunteers. *Psychopharmacology (Berl)* 2015; **232**: 785–794.
- Dolder PC, Schmid Y, Haschke M, Rentsch KM, Liechti ME. Pharmacokinetics and concentration-effect relationship of oral LSD in humans. *Int J Neuropsychopharmacol* 2015; **19**: pyv072.

- 7 Strajhar P, Schmid Y, Liakoni E, Dolder PC, Rentsch KM, Kratschmar DV et al. Acute effects of LSD on circulating steroid levels in healthy subjects. *J Neuroendocrinol* 2016; **28**: 12374.
- 8 Johnson MW, Garcia-Romeu A, Cosimano MP, Griffiths RR. Pilot study of the 5-HT_{2A} agonist psilocybin in the treatment of tobacco addiction. *J Psychopharmacol* 2014; **28**: 983–992.
- 9 Bogenschütz MP, Forchimes AA, Pommy JA, Wilcox CE, Barbosa PC, Strassman RJ. Psilocybin-assisted treatment for alcohol dependence: a proof-of-concept study. *J Psychopharmacol* 2015; **29**: 289–299.
- 10 Grob CS, Danforth AL, Chopra GS, Hagerty M, McKay CR, Halberstadt AL et al. Pilot study of psilocybin treatment for anxiety in patients with advanced-stage cancer. *Arch Gen Psychiatry* 2011; **68**: 71–78.
- 11 Carhart-Harris RL, Bolstridge M, Rucker J, Day CM, Erritzoe D, Kaelin M et al. Psilocybin with psychological support for treatment-resistant depression: an open-label feasibility study. *Lancet Psychiatry* 2016; **3**: 619–627.
- 12 Gasser P, Kirchner K, Passie T. LSD-assisted psychotherapy for anxiety associated with a life-threatening disease: a qualitative study of acute and sustained subjective effects. *J Psychopharmacol* 2015; **29**: 57–68.
- 13 Adolphs R. Fear, faces, and the human amygdala. *Curr Opin Neurobiol* 2008; **18**: 166–172.
- 14 Fusar-Poli P, Placentino A, Carletti F, Landi P, Allen P, Surguladze S et al. Functional atlas of emotional faces processing: a voxel-based meta-analysis of 105 functional magnetic resonance imaging studies. *J Psychiatry Neurosci* 2009; **34**: 418–432.
- 15 Bedi G, Phan KL, Angstadt M, de Wit H. Effects of MDMA on sociability and neural response to social threat and social reward. *Psychopharmacology (Berl)* 2009; **207**: 73–83.
- 16 Thirion B, Pinel P, Meriaux S, Roche A, Dehaene S, Poline JB. Analysis of a large fMRI cohort: Statistical and methodological issues for group analyses. *Neuroimage* 2007; **35**: 105–120.
- 17 Schmidt A, Borgwardt S, Gerber H, Wiesbeck GA, Schmid O, Riecher-Rössler A et al. Acute effects of heroin on negative emotional processing: relation of amygdala activity and stress-related responses. *Biol Psychiatry* 2014; **76**: 289–296.
- 18 Domes G, Heinrichs M, Kumbier E, Grossmann A, Hauenstein K, Herpertz SC. Effects of intranasal oxytocin on the neural basis of face processing in autism spectrum disorder. *Biol Psychiatry* 2013; **74**: 164–171.
- 19 Passie T, Halpern JH, Stichtenoth DO, Emrich HM, Hintzen A. The pharmacology of lysergic acid diethylamide: a review. *CNS Neurosci Ther* 2008; **14**: 295–314.
- 20 Dolder PC, Schmid Y, Müller F, Borgwardt S, Liechi ME. LSD acutely impairs fear recognition and enhances emotional empathy and sociality. *Neuropsychopharmacology* 2016; **41**: 2638–2646.
- 21 Kometer M, Schmidt A, Bachmann R, Studerus E, Seifritz E, Vollenweider FX. Psilocybin biases facial recognition, goal-directed behavior, and mood state toward positive relative to negative emotions through different serotonergic subreceptors. *Biol Psychiatry* 2012; **72**: 898–906.
- 22 Schmidt A, Kometer M, Bachmann R, Seifritz E, Vollenweider F. The NMDA antagonist ketamine and the 5-HT agonist psilocybin produce dissociable effects on structural encoding of emotional face expressions. *Psychopharmacology (Berl)* 2013; **225**: 227–239.
- 23 Kraehenmann R, Preller KH, Scheidegger M, Pokorny T, Bosch OG, Seifritz E et al. Psilocybin-induced decrease in amygdala reactivity correlates with enhanced positive mood in healthy volunteers. *Biol Psychiatry* 2015; **78**: 572–581.
- 24 Morris JS, Frith CD, Perrett DI, Rowland D, Young AW, Calder AJ et al. A differential neural response in the human amygdala to fearful and happy facial expressions. *Nature* 1996; **383**: 812–815.
- 25 Blair RJ, Morris JS, Frith CD, Perrett DI, Dolan RJ. Dissociable neural responses to facial expressions of sadness and anger. *Brain* 1999; **122**(Pt 5): 883–893.
- 26 Wright CI, Fischer H, Whalen PJ, McNerney SC, Shin LM, Rauch SL. Differential prefrontal cortex and amygdala habituation to repeatedly presented emotional stimuli. *Neuroreport* 2001; **12**: 379–383.
- 27 Phillips ML, Medford N, Young AW, Williams L, Williams SC, Bullmore ET et al. Time courses of left and right amygdalar responses to fearful facial expressions. *Hum Brain Mapp* 2001; **12**: 193–202.
- 28 Sergerie K, Chochol C, Armony JL. The role of the amygdala in emotional processing: a quantitative meta-analysis of functional neuroimaging studies. *Neurosci Biobehav Rev* 2008; **32**: 811–830.
- 29 Baas D, Aleman A, Kahn RS. Lateralization of amygdala activation: a systematic review of functional neuroimaging studies. *Brain Res Brain Res Rev* 2004; **45**: 96–103.
- 30 Wager TD, Phan KL, Liberzon I, Taylor SF. Valence, gender, and lateralization of functional brain anatomy in emotion: a meta-analysis of findings from neuroimaging. *Neuroimage* 2003; **19**: 513–531.
- 31 Hensler JG. Serotonergic modulation of the limbic system. *Neurosci Biobehav Rev* 2006; **30**: 203–214.
- 32 Asan E, Steinke M, Lesch KP. Serotonergic innervation of the amygdala: targets, receptors, and implications for stress and anxiety. *Histochem Cell Biol* 2013; **139**: 785–813.
- 33 Bombardi C. Neuronal localization of the 5-HT₂ receptor family in the amygdaloid complex. *Front Pharmacol* 2014; **5**: 68.
- 34 Murphy SE, Norbury R, O'Sullivan U, Cowen PJ, Harmer CJ. Effect of a single dose of citalopram on amygdala response to emotional faces. *Br J Psychiatry* 2009; **194**: 535–540.
- 35 Harmer CJ, Mackay CE, Reid CB, Cowen PJ, Goodwin GM. Antidepressant drug treatment modifies the neural processing of nonconscious threat cues. *Biol Psychiatry* 2006; **59**: 816–820.
- 36 Godlewska BR, Norbury R, Selvaraj S, Cowen PJ, Harmer CJ. Short-term SSRI treatment normalises amygdala hyperactivity in depressed patients. *Psychol Med* 2012; **42**: 2609–2617.
- 37 Sheline YI, Barch DM, Donnelly JM, Ollinger JM, Snyder AZ, Mintun MA. Increased amygdala response to masked emotional faces in depressed subjects resolves with antidepressant treatment: an fMRI study. *Biol Psychiatry* 2001; **50**: 651–658.
- 38 Bocchio M, McHugh SB, Bannerman DM, Sharp T, Capogna M. Serotonin, amygdala and fear: assembling the puzzle. *Front Neural Circuits* 2016; **10**: 24.
- 39 Ghashghaie HT, Hilgetag CC, Barbas H. Sequence of information processing for emotions based on the anatomic dialogue between prefrontal cortex and amygdala. *Neuroimage* 2007; **34**: 905–923.
- 40 Etkin A, Egner T, Kalisch R. Emotional processing in anterior cingulate and medial prefrontal cortex. *Trends Cogn Sci* 2011; **15**: 85–93.
- 41 Robinson OJ, Charney DR, Overstreet C, Vytal K, Grillon C. The adaptive threat bias in anxiety: amygdala-dorsomedial prefrontal cortex coupling and aversive amplification. *Neuroimage* 2012; **60**: 523–529.
- 42 Robinson OJ, Krinsky M, Lieberman L, Allen P, Vytal K, Grillon C. Towards a mechanistic understanding of pathological anxiety: the dorsal medial prefrontal-amygdala 'aversive amplification' circuit in unmedicated generalized and social anxiety disorders. *Lancet Psychiatry* 2014; **1**: 294–302.
- 43 Robinson OJ, Krinsky M, Lieberman L, Vytal K, Ernst M, Grillon C. Anxiety-potentiated amygdala-medial frontal coupling and attentional control. *Transl Psychiatry* 2016; **6**: e833.
- 44 Robinson OJ, Overstreet C, Allen PS, Letkiewicz A, Vytal K, Pine DS et al. The role of serotonin in the neurocircuitry of negative affective bias: serotonergic modulation of the dorsal medial prefrontal-amygdala 'aversive amplification' circuit. *Neuroimage* 2013; **78**: 217–223.
- 45 Griffiths RR, Johnson MW, Richards WA, Richards BD, McCann U, Jesse R. Psilocybin occasioned mystical-type experiences: immediate and persisting dose-related effects. *Psychopharmacology (Berl)* 2011; **218**: 649–665.
- 46 Groenewold NA, Opmeer EM, de Jonge P, Aleman A, Costafreda SG. Emotional valence modulates brain functional abnormalities in depression: evidence from a meta-analysis of fMRI studies. *Neurosci Biobehav Rev* 2013; **37**: 152–163.
- 47 Etkin A, Wager TD. Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. *Am J Psychiatry* 2007; **164**: 1476–1488.
- 48 Mithoefer MC, Wagner MT, Mithoefer AT, Jerome L, Doblin R. The safety and efficacy of (+/-)-3,4-methylenedioxymethamphetamine-assisted psychotherapy in subjects with chronic, treatment-resistant posttraumatic stress disorder: the first randomized controlled pilot study. *J Psychopharmacol* 2011; **25**: 439–452.
- 49 Oehen P, Traber R, Widmer V, Schnyder U. A randomized, controlled pilot study of MDMA (±3,4-methylenedioxymethamphetamine)-assisted psychotherapy for treatment of resistant, chronic post-traumatic stress disorder (PTSD). *J Psychopharmacol* 2013; **27**: 40–52.
- 50 Mithoefer MC, Wagner MT, Mithoefer AT, Jerome L, Martin SF, Yazar-Klosinski B et al. Durability of improvement in post-traumatic stress disorder symptoms and absence of harmful effects or drug dependency after 3,4-methylenedioxymethamphetamine-assisted psychotherapy: a prospective long-term follow-up study. *J Psychopharmacol* 2013; **27**: 28–39.
- 51 Osorio Fde L, Sanches RF, Macedo LR, Santos RG, Maia-de-Oliveira JP, Wichert-Ana L et al. Antidepressant effects of a single dose of ayahuasca in patients with recurrent depression: a preliminary report. *Rev Bras Psiquiatr* 2015; **37**: 13–20.
- 52 Vollenweider FX, Kometer M. The neurobiology of psychedelic drugs: implications for the treatment of mood disorders. *Nat Rev Neurosci* 2010; **11**: 642–651.



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3.6 Publication 6

Development and validation of an LC-MS/MS method to quantify LSD, iso-LSD, 2-oxo-3-hydroxy LSD, and nor-LSD and identify novel metabolites in plasma samples in a controlled clinical trial

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RESEARCH ARTICLE

Development and validation of an LC-MS/MS method to quantify lysergic acid diethylamide (LSD), iso-LSD, 2-oxo-3-hydroxy-LSD, and nor-LSD and identify novel metabolites in plasma samples in a controlled clinical trial

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Background: Lysergic acid diethylamide (LSD) is a widely used recreational drug. The aim of this study was to develop and validate a liquid chromatography tandem mass spectrometry (LC-MS/MS) method for the quantification of LSD, iso-LSD, 2-oxo-3-hydroxy LSD (O-H-LSD), and nor-LSD in plasma samples from 24 healthy subjects after controlled administration of 100 µg LSD in a clinical trial. In addition, metabolites that have been recently described in in vitro studies, including lysergic acid monoethylamide (LAE), lysergic acid ethyl-2-hydroxyethylamide (LEO), 2-oxo-LSD, trioxylated-LSD, and 13/14-hydroxy-LSD, should be identified.

Methods: Separation of LSD and its metabolites was achieved on a reversed phase chromatography column after turbulent-flow online extraction. For the identification and quantification, a triple-stage quadrupole LC-MS/MS instrument was used.

Results: The validation data showed slight matrix effects for LSD, iso-LSD, O-H-LSD, or nor-LSD. Mean intraday and interday accuracy and precision were 105%/4.81% and 105%/4.35% for LSD, 98.7%/5.75% and 99.4%/7.21% for iso-LSD, 106%/4.54% and 99.4%/7.21% for O-H-LSD, and 107%/5.82% and 102%/5.88% for nor-LSD, respectively. The limit of quantification was 0.05 ng/mL for LSD, iso-LSD, and nor-LSD and 0.1 ng/mL for O-H-LSD. The limit of detection was 0.01 ng/mL for all compounds.

Conclusion: The method described herein was accurate, precise, and the calibration range within the range of expected plasma concentrations. LSD was quantified in the plasma samples of the 24 subjects of the clinical trial, whereas iso-LSD, O-H-LSD, nor-LSD, LAE, LEO, 13/14-hydroxy-LSD, and 2-oxo-LSD could only sporadically be detected but were too low for quantification.

KEYWORDS

controlled study, LC-MS, LSD, lysergic acid diethylamide, metabolism, plasma

1 | INTRODUCTION

Lysergic acid diethylamide (LSD) is a psychoactive substance that alters states of consciousness and perception. Its psychedelic effects made it popular as a recreational drug, especially in the 1960s and 1970s, but LSD is still widely used today.¹ In addition, LSD has been

reintroduced in psychiatric research²⁻¹⁶ and investigated as an adjunct to psychotherapy.^{17,18} Therefore, information about its metabolism and pharmacokinetics after controlled intake has received increasing interest. Doses that were used in recent clinical studies ranged from 75 µg, i.v.,²⁻¹¹ to 200 µg, p.o.,^{12-15,19} resulting in low blood and urine concentrations.^{12,20} Dolder et al. and Steuer et al. recently showed

that LSD and its main urinary metabolite 2-oxo-3-hydroxy-LSD (O-H-LSD) were detectable in plasma after controlled intake of 200 µg LSD in 16 healthy subjects^{12,20} and clinical toxicological cases of acute LSD overdose.²¹ Studies of in vitro metabolism have further identified lysergic acid monoethylamide (LAE), lysergic acid ethyl-2-hydroxyethylamide (LEO), 2-oxo-LSD, nor-LSD, trioxylated-LSD, and 13/14-hydroxy-LSD as glucuronides,^{22,23} but no systematic information is available regarding their presence in human plasma after controlled intake of LSD. However, recent investigations confirmed the presence of 2-oxo-LSD and 13/14-hydroxy-LSD (glucuronides) in plasma samples after controlled intake of 200 µg LSD.²⁰ The aim of this study was to develop a sensitive turboflow liquid chromatography tandem mass spectrometry (LC-MS/MS) method to quantify LSD, iso-LSD, O-H-LSD, and nor-LSD and potentially identify LAE, LEO, 2-oxo-LSD, trioxylated-LSD, and 13/14-hydroxy-LSD (glucuronides) in human plasma samples. The method was developed using a triple-stage quadrupole LC-MS/MS instrument in selected reaction monitoring (SRM) mode after atmospheric pressure ionization (APCI). Our method was established and successfully applied to the analysis of plasma samples from healthy volunteers after the intake of 100 µg LSD in a controlled clinical study.

2 | MATERIALS AND METHODS

2.1 | Chemicals and reagents

Acetonitrile, acetone, methanol, 2-propanol, formic acid, and acetic acid with high-performance liquid chromatography (HPLC)-grade purity were all purchased from Merck (Darmstadt, Germany). HPLC-grade ammonium acetate and ammonium carbonate were obtained from Merck. Distilled water was obtained from an in-house installed purifier (ELGA, Bucks, UK). Drug-free plasma samples (containing lithium-heparin as an anticoagulant) serving as negative control, and blank matrices were obtained from coworkers. LSD and LSD-d₃ as 1 mg/mL reference standards in acetonitrile were obtained from Lipomed (Arlesheim, Switzerland). O-H-LSD and iso-LSD as 0.1 mg/mL reference standards in acetonitrile were obtained from Cerilliant (Round Rock, TX, USA). Nor-LSD in powder form was obtained from Toronto Research Chemicals (Toronto, Canada). The non-commercially available metabolites LAE, LEO, 2-oxo-LSD, trioxylated-LSD, and 13/14-hydroxy-LSD (glucuronides) were extracted from pooled 24-h urine samples as described in Results section.

2.2 | LC-MS/MS analysis

2.2.1 | Equipment

The HPLC system (Transcend TLX1 HPLC; Thermo Scientific, Basel, Switzerland) consisted of two Accela 1250 pumps for loading and eluting. The autosampler and sample extraction system were controlled by the Aria MX 2.1 software (Thermo Scientific). A cyclone P turboflow column (Thermo Scientific) was used for extraction, and a Zorbax Eclipse XDB-C8 column (Agilent, Santa Clara, CA, USA) was

used for chromatographic separation. The online extraction system was coupled to a TSQ Endura triple-stage mass spectrometer (Thermo Scientific) using APCI in positive mode because of its better performance with regard to matrix effects.^{24,25}

2.2.2 | Liquid chromatography method

For LC, three mobile phases were used in gradient mode for extraction and analytical chromatography. Mobile phase A consisted of 20 mmol/L ammonium acetate in water and 0.1% formic acid. Mobile phase B consisted of 20 mmol/L ammonium acetate in methanol and acetonitrile (1:1) that contained 0.1% formic acid. Mobile phase C was an organic mixture of acetonitrile, acetone, and 2-propanol (1:1:1). Chromatography was run in isocratic mode with 70% mobile phase A and 30% mobile phase B, with a run time of 11 minutes and four additional minutes for flushing and equilibration using mobile phase C.

2.2.3 | Mass spectrometry conditions

The positive ion discharge current was set to 5 µA. The vaporizer temperature was optimized to 400°C. Sheath and auxiliary gas provided the best results, with flow rates of 15 and 5 arbitrary units, respectively. The temperature of the ion transfer tube was set to 300°C. The system was tuned and optimized for the detection of LSD. LSD and its metabolites were detected using SRM of the two to three most intense ion transitions. Analytes were identified when quantifier and qualifier ions were present within the given retention time. Structures, transitions, and respective collision energies are shown in Figure 1.

2.3 | Standard solutions

Stock solutions that contained 100 µg/mL LSD, 100 µg/mL LSD-d₃, 10 µg/mL iso-LSD, 10 µg/mL O-H-LSD, or 10 µg/mL nor-LSD in acetonitrile were prepared and stored in light-protected brown glass vials at -20°C. All of the solutions were prepared in duplicate to have different sets for quality control (QC) and calibration samples. Working solutions of each analyte at 0.1 µg/mL in purified water/acetonitrile were used for the preparation of QC and calibration samples and matrix and selectivity experiments. Because of the instability of LSD and to minimize possible degradation by various freeze-thaw cycles, 1 mL aliquots of stock and working solutions were prepared.

2.4 | Sample preparation

Study samples were sorted according to drug condition (LSD or placebo) and subject (S1-24). Calibrators, controls and subject samples were thawed once, and 100 µL aliquots was taken to minimize the freeze-thaw cycles. To 100 µL of plasma, 110 µL of an acetonitrile/LSD-d₃ solution (0.01 µg/mL) was added. The samples were then vigorously vortexed and centrifuged for 10 minutes at 13 200 g, and the supernatant was then transferred to 96-well plates.

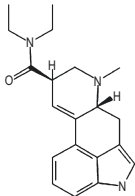
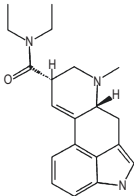
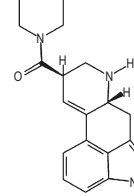
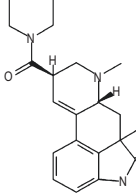
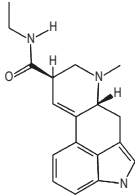
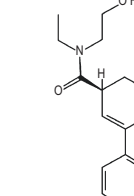
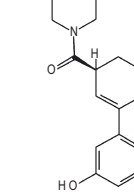
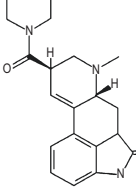
<p>Lysergic-acid-diethylamide (LSD)</p>  <p>Retention time: 8.50 min</p> <p>Transitions: Collision Energy (V)</p> <p>324 → 223 24</p> <p>324 → 208 31</p> <p>324 → 197 24</p>	<p>Iso-LSD</p>  <p>Retention time: 9.10 min</p> <p>Transitions: Collision Energy (V)</p> <p>324 → 223 24</p> <p>324 → 208 31</p> <p>324 → 197 24</p>	<p>Nor-LSD</p>  <p>Retention time: 8.85 min</p> <p>Transitions: Collision Energy (V)</p> <p>310 → 237 21</p> <p>310 → 209 24</p> <p>310 → 192 39</p>	<p>2-oxo-3-hydroxy-LSD (O-H-LSD)</p>  <p>Retention time: 4.52 min</p> <p>Transitions: Collision Energy (V)</p> <p>356 → 338 14</p> <p>356 → 265 17</p> <p>356 → 237 22</p>
<p>Lysergic-acid-monoethylamide (LAE)</p>  <p>Retention time: 5.31 min</p> <p>Transitions: Collision Energy (V)</p> <p>296 → 253 24</p> <p>296 → 223 24</p> <p>296 → 208 30</p>	<p>Lysergic-acid-ethyl-hydroxyethylamide (LEO)</p>  <p>Retention time: 5.14 min</p> <p>Transitions: Collision Energy (V)</p> <p>340 → 223 24</p> <p>340 → 239 22</p>	<p>13/14-hydroxy-LSD</p>  <p>Retention time: 4.54 min</p> <p>Transitions: Collision Energy (V)</p> <p>358 → 340 24</p> <p>358 → 239 22</p>	<p>2-oxo-LSD</p>  <p>Retention time: 5.35 min</p> <p>Transitions: Collision Energy (V)</p> <p>340 → 223 24</p> <p>340 → 208 30</p>

FIGURE 1 Structure, retention time, ion transitions, and collision energies of lysergic acid diethylamide (LSD) and selected metabolites

2.5 | Experiments

2.5.1 | Calibration

Six calibration standards were prepared by spiking plasma samples with LSD, iso-LSD, and nor-LSD to concentrations of 0.05, 0.1, 0.5, 1, 5, and 10 ng/mL plus blank (matrix only) and zero sample (matrix plus internal standard). Five calibrators were used for O-H-LSD with concentrations of 0.05, 0.1, 0.5, 1, 5, and 10 ng/mL plus blank (matrix only) and zero sample (matrix plus internal standard). The highest calibration point in plasma was adopted from our previously developed method and pharmacokinetic-pharmacodynamic data.^{12,21} The calibration curves were linearly fitted using a weighting factor of $1/x^2$.

2.5.2 | Selectivity

Following U.S. Food and Drug Administration validation guidelines,²⁶ we collected plasma samples from six different healthy volunteers and tested them for interference to establish selectivity. We further analyzed samples from the placebo condition to confirm the absence of LSD.

2.5.3 | Matrix effects and recovery

Matrix effects, recovery, and process efficiency were measured and calculated according to Matuszewski et al.²⁷ In regard of the vulnerability to light and air and because of the online extraction that was

used in the present method, the extraction step comprised only protein precipitation. All of the samples were processed through the turbulent-flow extraction column. Five plasma samples were spiked to concentrations between 0.05 and 10 ng/mL for LSD, iso-LSD, O-H-LSD, and nor-LSD. The samples were measured before and after extraction and in neat solution. The peak areas of the spiked samples after extraction were then compared with the area of the spiked mobile phase to calculate matrix effects. Recovery values were calculated as the areas of standards that were spiked before extraction divided by the areas of standards that were spiked after extraction. The process efficiency was adopted from Matuszewski et al.²⁷ and calculated as the ratio between the area of the standard spiked before extraction and the areas of the standard in neat solution.

2.5.4 | Stability

The determination of long-term stability was based on Li et al. and Klette et al., in which LSD is regarded as stable under storage conditions of -20°C .^{28,29} However, LSD is known to be very unstable and vulnerable to air, light, and heat. Even ambient temperature ($20\text{--}25^{\circ}\text{C}$) and normal light conditions can lead to a decrease in LSD concentrations. Therefore, we assessed bench-top stability and autosampler stability with multiple measurements of calibration and QC samples within 24 h. For autosampler stability, the samples were kept in light-protected, sealed, 96-well deep-well plates at 4°C in the autosampler until injection. During the study, the samples were drawn through an intravenous catheter into lithium-heparin tubes and directly

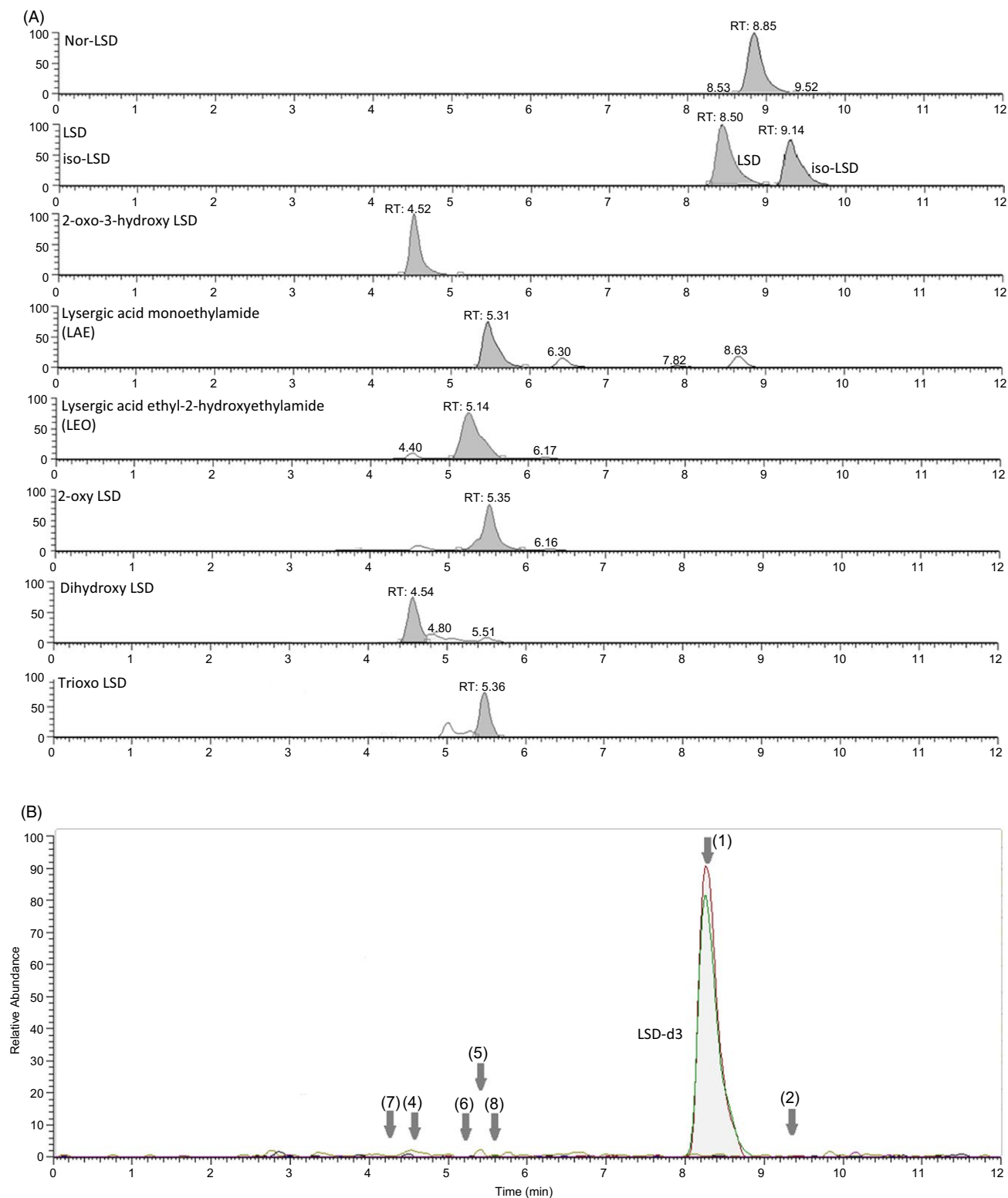


FIGURE 2 (A) Chromatogram of selected metabolites. Lysergic acid diethylamide (LSD), iso-LSD, nor-LSD, and 2-oxo-3-hydroxy-LSD are spiked at 1 ng/mL in plasma; the concentration of lysergic-acid monoethylamide, lysergic-acid-ethyl-2-hydroxyethylamide, 13/14-hydroxy-LSD, and 2-oxo-LSD is unknown. (B) Chromatogram of a healthy volunteer 4 h after administration of 100 µg LSD. Arrows are indicating peaks of LSD (1), iso-LSD (2), nor-LSD (3) and 2-oxo-3-hydroxy-LSD (4), lysergic-acid monoethylamide (5), lysergic-acid-ethyl-2-hydroxyethylamide (6), 13/14-hydroxy-LSD (7), and 2-oxo-LSD (8)

centrifuged, and the plasma was stored at -20°C at the study site before transferring to the laboratory for analysis. Due to the known vulnerability of LSD, calibrators and quality controls were freshly weighted every week and single aliquots were stored at -20°C . A new calibration was run every day and with every study subject.

2.5.5 | Lower limits of detection and quantification

Drug-free plasma samples were spiked with different concentrations of LSD, iso-LSD, O-H-LSD, and nor-LSD for determination of the limit of quantification (LOQ) and the limit of detection (LOD). The LOQ concentrations had to give a response at least five times greater than the blank. In addition, precision had to be $<20\%$, and accuracy had to be 80% - 120% using at least five determinations per matrix and concentration. The LOD concentration was determined as the lowest discriminable peak in the region of a signal-to-noise ratio greater than five.

2.5.6 | Carryover

For the determination of the carryover, different blank plasma samples were run between patient samples, highest calibrations, and quality controls.

2.5.7 | Reproducibility

According to U.S. Food and Drug Administration guidelines,²⁶ the reproducibility of quantification was determined by measuring each QC sample five times in 1 day to establish intraday precision and accuracy. Each QC sample was also measured for five consecutive days to determine interday precision and accuracy. All of the values had to meet the criteria of a coefficient of variation (CV) $<15\%$, response $<20\%$ at the LOQ, and accuracy of 80% - 120% . To demonstrate the accuracy and precision of the method, we used three QCs (low, medium, and high). The QC concentrations were 0.05, 1, and 10 ng/mL

for LSD, iso-LSD, and nor-LSD, and 0.1, 1, and 10 ng/mL for O-H-LSD, respectively.

3 | RESULTS

Lysergic acid diethylamide, LSD- d_3 , iso-LSD, and the metabolites nor-LSD, LAE, LEO, 2-oxo-LSD, trioxylated-LSD, and 13/14-hydroxy-LSD (glucuronides) eluted between 4 and 11 minutes. The chromatographic separation of spiked samples and selected metabolites is depicted in Figure 2A, and the chromatogram of a subject's sample 4 h after LSD administration is presented in Figure 2B.

3.1 | Selectivity

None of the six plasma samples showed any interference within the measured mass range and time frame (Figure 3). Furthermore, none of the measured plasma samples from the placebo condition showed any interference.

3.2 | Matrix effects and recovery

The plasma matrix effects were 125% for LSD, 119% for iso-LSD, 103% for O-H-LSD, and 118% for nor-LSD at concentrations of 10 ng/mL, consistent with a slight ion enhancement for LSD, iso-LSD, and nor-LSD. Recoveries were calculated as 70%-90% for all substances at 10 ng/mL. Process efficiencies were 113% for LSD, 86% for iso-LSD, 77% for O-H-LSD, and 93% for nor-LSD.

3.3 | Stability

The concentrations of the processed samples decreased up to $\sim 60\%$ within 24 hours at ambient temperature (20 - 23°C). The concentrations of the extracted and sealed plasma samples that

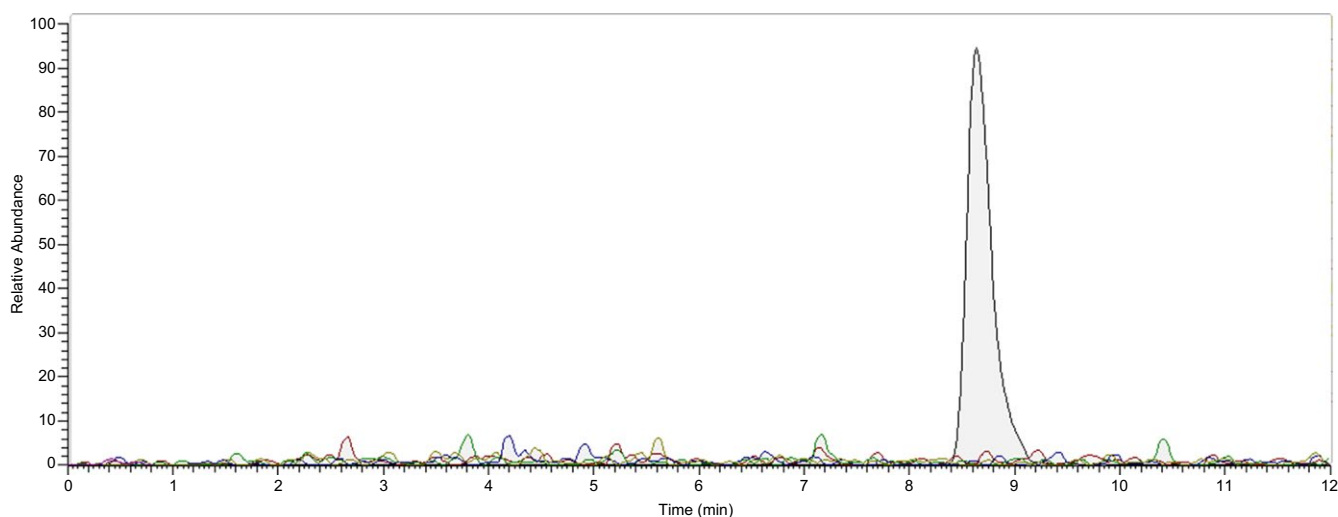


FIGURE 3 Chromatogram of 6 blank plasma samples from six different subjects, and a blank sample containing lysergic acid diethylamide- d_3

were stored within the closed autosampler at 4°C were stable up to 24 hours.

3.4 | Lower limits of detection and quantification

The LOQ was 0.05 ng/mL for LSD, iso-LSD, and nor-LSD. For O-H-LSD, the respective concentration was 0.1 ng/mL. The LODs were 0.01 ng/mL for all compounds.

3.5 | Carryover

No carryover was found for LSD, iso-LSD, O-H-LSD, or nor-LSD in the plasma samples. Despite these results as a preventive measure, a consecutive blank was always run after the highest calibrator (10 ng/mL) and QC (10 ng/mL) during method development and the measurement of the study samples.

3.6 | Linearity

Calibration curves in plasma were linear over the respective calibration ranges, with a mean correlation coefficient (R^2) of 0.99. The calibration curves (mean \pm SEM) are shown in Figure 4.

3.7 | Reproducibility

All of the substances fulfilled the accuracy and precision criteria. The mean intraday accuracy and precision were 105% and 4.81% for LSD, 98.7% and 5.75% for iso-LSD, 106% and 4.54% for O-H-LSD, and 107% and 5.82% for nor-LSD, respectively. The mean interday accuracy and precision were 105% and 4.35% for LSD, 99.4% and 7.21% for iso-LSD, 99.4% and 7.21% for O-H-LSD, and 102% and 5.88% for nor-LSD, respectively.

3.8 | Identification of non-commercially available LSD metabolites

Lysergic acid diethylamide metabolites were extracted by liquid-liquid extraction from pooled LSD-positive 24-h urine samples (8 L) to reach high concentrations. One part of the concentrated metabolites was kept for eventual quantification, and the second part was extracted using industrial separation by automated thin-layer chromatography and purification. Separation was performed with generous support from Camag (Muttens, Switzerland). Parent masses and selected transitions for LC-MS were adopted from Cai et al.²² and Canezin et al.²³ and replicated by injecting a mixture of the concentrated, extracted

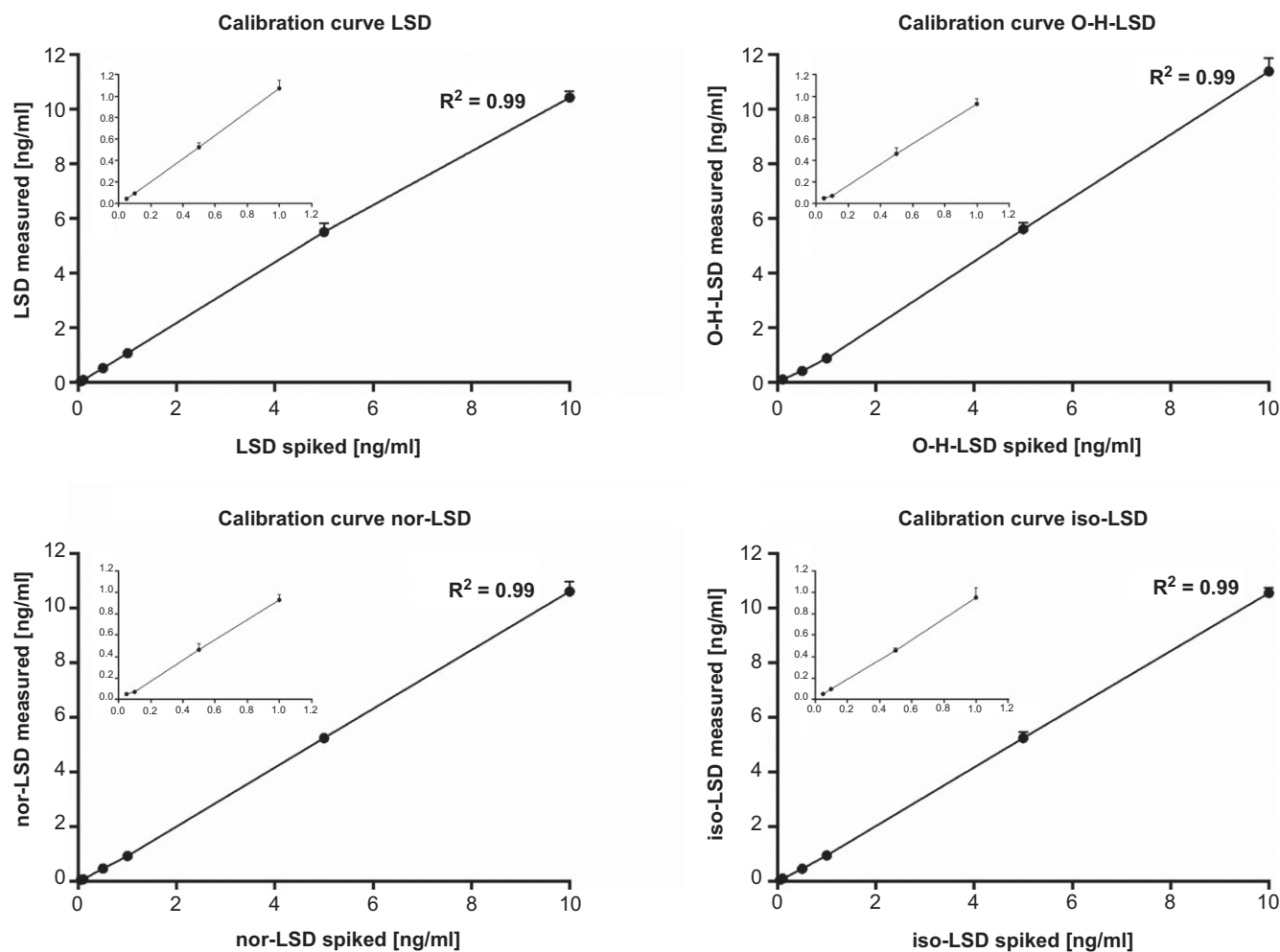


FIGURE 4 Calibration curves of lysergic acid diethylamide (LSD), iso-LSD, nor-LSD, and 2-oxo-3-hydroxy-LSD in human plasma

metabolites. All of the identified metabolites from concentrated urine samples (LAE, LEO, 2-oxo-LSD, trioxylated-LSD, and 13/14-hydroxy-LSD) were added to the quantification method before validation, for qualitative screening of the study samples.

3.9 | Samples

LSD (100 µg) and placebo were administered to 24 healthy subjects (12 women, 12 men) in a double-blind, randomized, placebo-controlled, cross-over study. The study was conducted in accordance with the Declaration of Helsinki and International Conference on Harmonization Guidelines in Good Clinical Practice (ICH-GCP) and approved by the Ethics Committee Northwest Switzerland and Swiss Federal Office for Public Health, Bern, Switzerland. The study was registered at ClinicalTrials.gov (NCT02308969). Plasma samples were collected at baseline and 1, 2, 3, 4, 6, 8, 10, 12, 16, and 24 h after LSD administration. Maximum LSD plasma concentrations of 1.3 ± 0.17 ng/mL (mean \pm SEM) were determined (Table 1). Nor-LSD could only be quantified in two subjects (3 and 4 hours post-administration), and LAE, LEO, 2-oxy LSD, and 13/14-hydroxy-LSD were detected in some of the samples. 13/14-hydroxy-LSD glucuronides were undetectable because they were cleaved during ionization. Detailed study descriptions, pharmacokinetic data, and pharmacokinetic-pharmacodynamic analyses will be published elsewhere.

4 | DISCUSSION AND CONCLUSION

With mean maximum plasma concentrations of LSD of ~ 1 ng/mL, the development of analytical methods for quantification remains a challenge and brings LC-MS technologies to their limits. For separation of the different analytes, various columns have been used. Especially, the separation of LSD and iso-LSD was challenging, and only achieved using the Zorbax Eclipse XDB-C8 column. However, the method was only developed to chromatographically separate LSD, iso-LSD, nor-LSD, and O-H-LSD. The non-commercially available metabolites were not available in sufficient amounts for extensive experiments. Further, to improve sensitivity, different sample preparation procedures (eg, liquid-liquid extraction using chlorobutane and tert-butyl-methylether) have been performed but have not led to significant changes in the LOQ. Considering the light and air sensitivity of LSD and the manual workload that is caused by liquid-liquid extraction or solid-phase extraction, simple and fast protein precipitation has been favored instead. APCI was equally to ESI regarding signal intensity but gave slightly better results regarding matrix effects and was therefore favored. Overall, quantifying plasma samples between 12 and 24 hours after LSD administration requires techniques that provide precise and sensitive measurements within the low picogram range. This poses a challenge to quantifying LSD concentrations and also makes it impossible to quantify or even identify new metabolites in plasma samples after controlled intake of 100 µg LSD. In our recent investigations,¹² we detected quantifiable plasma levels of O-H-LSD

TABLE 1 Measured plasma concentrations (C_{\max}) and the corresponding time points (T_{\max}) following oral administration of 100 µg lysergic acid diethylamide in 24 healthy subjects

Subject	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
C_{\max} (ng/mL)	1.7	1.2	1.2	2.0	2.1	1.5	1.4	1.9	3.3	2.0	0.8	3.2	0.8	0.9	1.4	0.8	1.9	1.3	0.3	1.8	1.9	1.1	1.4	1.2
T_{\max} (h)	1	1	2	1	3	1	2	2	1	2	3	1	1	2	2	1	1	2	1	2	2	3	2	3

after the administration of 200 µg LSD. Steuer et al.²⁰ additionally identified O-H-LSD and 13/14-hydroxy-LSD (glucuronides). We did not expect to detect quantifiable concentrations of LSD metabolites after the administration of 100 µg LSD. The metabolites did not reach the LOD of our or other methods. Nevertheless, we sporadically detected the presence of metabolites in some plasma samples and could confirm the presence of O-H-LSD, nor-LSD, LEO, LAE, and 13/14-hydroxy-LSD in plasma. To investigate the metabolism of LSD more comprehensively, further studies that use higher doses of LSD are required and metabolites need to be commercially available to develop comprehensive analytical methods for their quantification.

REFERENCES

1. Passie T, Halpern JH, Stichtenoth DO, et al. The pharmacology of lysergic acid diethylamide: a review. *CNS Neurosci Ther.* 2008;14:295-314.
2. Carhart-Harris RL, Kaelen M, Whalley MG, et al. LSD enhances suggestibility in healthy volunteers. *Psychopharmacology.* 2015;232:785-794.
3. Kaelen M, Barrett FS, Roseman L, et al. LSD enhances the emotional response to music. *Psychopharmacology.* 2015;232:3607-3614.
4. Carhart-Harris RL, Kaelen M, Bolstridge M, et al. The paradoxical psychological effects of lysergic acid diethylamide (LSD). *Psychol Med.* 2016;46:1379-1390.
5. Carhart-Harris RL, Muthukumaraswamy S, Roseman L, et al. Neural correlates of the LSD experience revealed by multimodal neuroimaging. *Proc Natl Acad Sci USA.* 2016;113:4853-4858.
6. Kaelen M, Roseman L, Kahan J, et al. LSD modulates music-induced imagery via changes in parahippocampal connectivity. *Eur Neuropsychopharmacol.* 2016;26:1099-1109.
7. Lebedev AV, Kaelen M, Lovden M, et al. LSD-induced entropic brain activity predicts subsequent personality change. *Hum Brain Mapp.* 2016;37:3203-3213.
8. Roseman L, Sereno MI, Leech R, et al. LSD alters eyes-closed functional connectivity within the early visual cortex in a retinotopic fashion. *Hum Brain Mapp.* 2016;37:3031-3040.
9. Speth J, Speth C, Kaelen M, et al. Decreased mental time travel to the past correlates with default-mode network disintegration under lysergic acid diethylamide. *J Psychopharmacol.* 2016;30:344-353.
10. Tagliazucchi E, Roseman L, Kaelen M, et al. Increased global functional connectivity correlates with LSD-induced ego dissolution. *Curr Biol.* 2016;26:1043-1050.
11. Terhune DB, Luke DP, Kaelen M, et al. A placebo-controlled investigation of synaesthesia-like experiences under LSD. *Neuropsychologia.* 2016;88:28-34.
12. Dolder PC, Schmid Y, Haschke M, et al. Pharmacokinetics and concentration-effect relationship of oral LSD in humans. *Int J Neuropsychopharmacol.* 2015;19:pyv072.
13. Dolder PC, Schmid Y, Mueller F, et al. LSD acutely impairs fear recognition and enhances emotional empathy and sociality. *Neuropsychopharmacology.* 2016;41:2638-2646.
14. Liechti ME, Dolder PC, Schmid Y. Alterations of consciousness and mystical-type experiences after acute LSD in humans. *Psychopharmacology.* 2017;234:1499-1510.
15. Strajhar P, Schmid Y, Liakoni E, et al. Acute effects of lysergic acid diethylamide on circulating steroid levels in healthy subjects. *J Neuroendocrinol.* 2016;28:12374.
16. Schmid Y, Enzler F, Gasser P, et al. Acute effects of lysergic acid diethylamide in healthy subjects. *Biol Psychiatry.* 2015;78:544-553.
17. Gasser P, Holstein D, Michel Y, et al. Safety and efficacy of lysergic acid diethylamide-assisted psychotherapy for anxiety associated with life-threatening diseases. *J Nerv Ment Dis.* 2014;202:513-520.
18. Gasser P, Kirchner K, Passie T. LSD-assisted psychotherapy for anxiety associated with a life-threatening disease: a qualitative study of acute and sustained subjective effects. *J Psychopharmacol.* 2015;29:57-68.
19. Schmid Y, Dolder PC, Liechti ME. Acute autonomic and psychotropic effects of LSD in healthy subjects in a placebo-controlled study. *Clin Toxicol.* 2015;53:359.
20. Steuer AE, Poetzsch M, Stock L, et al. Development and validation of an ultra-fast and sensitive microflow liquid chromatography-tandem mass spectrometry (MFLC-MS/MS) method for quantification of LSD and its metabolites in plasma and application to a controlled LSD administration study in humans. *Drug Test Anal.* 2016. <https://doi.org/10.1002/dta.2042>
21. Dolder PC, Liechti ME, Rentsch KM. Development and validation of a rapid turboflow LC-MS/MS method for the quantification of LSD and 2-oxo-3-hydroxy LSD in serum and urine samples of emergency toxicological cases. *Anal Bioanal Chem.* 2015;407:1577-1584.
22. Cai J, Henion J. Elucidation of LSD in vitro metabolism by liquid chromatography and capillary electrophoresis coupled with tandem mass spectrometry. *J Anal Toxicol.* 1996;20:27-37.
23. Canezini J, Cailleux A, Turcant A, et al. Determination of LSD and its metabolites in human biological fluids by high-performance liquid chromatography with electrospray tandem mass spectrometry. *J Chromatogr B Biomed Sci Appl.* 2001;765:15-27.
24. Caban M, Migowska N, Stepnowski P, et al. Matrix effects and recovery calculations in analyses of pharmaceuticals based on the determination of β -blockers and β -agonists in environmental samples. *J Chromatogr A.* 2012;1258:117-127.
25. Dams R, Huestis MA, Lambert WE, et al. Matrix effect in bioanalysis of illicit drugs with LC-MS/MS: influence of ionization type, sample preparation, and biofluid. *J Am Soc Mass Spectrom.* 2003;14:1290-1294.
26. Food and Drug Administration. 2015. Analytical Procedures and Methods Validation for Drugs and Biologics: Guidance for Industry. <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM386366.pdf>. Accessed December 6, 2016.
27. Matuszewski BK, Constanzer ML, Chavez-Eng CM. Strategies for the assessment of matrix effect in quantitative bioanalytical methods based on HPLC-MS/MS. *Anal Chem.* 2003;75:3019-3030.
28. Klette KL, Horn CK, Stout PR, et al. LC-MS analysis of human urine specimens for 2-oxo-3-hydroxy LSD: method validation for potential interferants and stability study of 2-oxo-3-hydroxy LSD under various storage conditions. *J Anal Toxicol.* 2002;26:193-200.
29. Li Z, McNally AJ, Wang H, et al. Stability study of LSD under various storage conditions. *J Anal Toxicol.* 1998;22:520-525.

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3.7 Publication 7

Pharmacokinetics and Pharmacodynamics of Lysergic Acid Diethylamide in Healthy Subjects

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Pharmacokinetics and Pharmacodynamics of Lysergic Acid Diethylamide in Healthy Subjects

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Abstract

Background and Objective Lysergic acid diethylamide (LSD) is used recreationally and in clinical research. The aim of the present study was to characterize the pharmacokinetics and exposure–response relationship of oral LSD. **Methods** We analyzed pharmacokinetic data from two published placebo-controlled, double-blind, cross-over studies using oral administration of LSD 100 and 200 µg in 24 and 16 subjects, respectively. The pharmacokinetics of the 100-µg dose is shown for the first time and data for the 200-µg dose were reanalyzed and included. Plasma concentrations of LSD, subjective effects, and vital signs were

repeatedly assessed. Pharmacokinetic parameters were determined using compartmental modeling. Concentration–effect relationships were described using pharmacokinetic–pharmacodynamic modeling.

Results Geometric mean (95% confidence interval) maximum plasma concentration values of 1.3 (1.2–1.9) and 3.1 (2.6–4.0) ng/mL were reached 1.4 and 1.5 h after administration of 100 and 200 µg LSD, respectively. The plasma half-life was 2.6 h (2.2–3.4 h). The subjective effects lasted (mean ± standard deviation) 8.2 ± 2.1 and 11.6 ± 1.7 h for the 100- and 200-µg LSD doses, respectively. Subjective peak effects were reached 2.8 and 2.5 h after administration of LSD 100 and 200 µg, respectively. A close relationship was observed between the LSD concentration and subjective response within subjects, with moderate counterclockwise hysteresis. Half-maximal effective concentration values were in the range of 1 ng/mL. No correlations were found between plasma LSD concentrations and the effects of LSD across subjects at or near maximum plasma concentration and within dose groups.

Conclusions The present pharmacokinetic data are important for the evaluation of clinical study findings (e.g., functional magnetic resonance imaging studies) and the interpretation of LSD intoxication. Oral LSD presented dose-proportional pharmacokinetics and first-order elimination up to 12 h. The effects of LSD were related to changes in plasma concentrations over time, with no evidence of acute tolerance.

Trial registration: NCT02308969, NCT01878942.

Electronic supplementary material The online version of this article (doi:10.1007/s40262-017-0513-9) contains supplementary material, which is available to authorized users.

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Key Points

The pharmacokinetics of lysergic acid diethylamide was dose proportional and the subjective effects were related to the time course of plasma concentrations within subjects, with no evidence of acute tolerance.

Between-subject differences in plasma concentrations of lysergic acid diethylamide did not predict the subjective response within a dose group and when plasma concentrations were above the half-maximal effective concentration of the response measures.

1 Introduction

Lysergic acid diethylamide (LSD) is the prototypical hallucinogen [1, 2]. Lysergic acid diethylamide has seen worldwide interest with regard to pharmacology, psychiatry, and society at large. Lysergic acid diethylamide continues to be used for recreational and personal purposes [3]. Additionally, considerable interest has been seen in its therapeutic potential [4–9], and experimental clinical research with LSD has recently been reinitiated [10–23]. However, basic pharmacokinetic information on LSD is largely missing. A small study in five male subjects reported a mean plasma elimination half-life of LSD of 175 min after intravenous administration (2 µg/kg) [24]. Another non-systematic study sampled blood after administration of LSD 160 µg in 13 subjects up to 2.5–5 h but because of sparse and short sampling could not derive pharmacokinetic parameters [25]. We recently reported the first pharmacokinetic data for orally administered LSD (200 µg) in 16 male and female subjects [23]. The concentrations of LSD were maximal after 1.5 h (median) and gradually declined to very low levels by 12 h, with an elimination half-life of 3.6 h [23].

Recent studies have reported the effects of LSD on various neuronal correlates of brain activation [12, 13, 16, 17]. However, plasma exposure and thus the actual presence of LSD in the body have not been documented in any of these studies to date. Unknown are the time point at which peak concentrations are reached and the actual or predicted concentrations of LSD at the time point at which pharmacodynamic outcomes were collected. Therefore, the primary goal of the present study was to describe the pharmacokinetics of a controlled administration of oral LSD by assessing the plasma concentration–time profile of two doses of LSD (100 and 200 µg). A

second goal was to link the plasma concentration changes over time within subjects to the acute subjective and autonomic effects of LSD to derive half-maximal effective concentration (EC₅₀) values using standard pharmacokinetic–pharmacodynamic modeling.

Researchers have correlated subjective drug effects with brain functional magnetic resonance imaging (fMRI) data [12, 13, 16, 17]. This approach likely detects significant correlations for subjective effects that show large between-subject variance but not for subjective effects of the substance that are consistently present in all subjects. Plasma concentrations of LSD have not been determined in any of the published LSD fMRI studies to date; therefore, it is unclear how LSD exposure in the body is linked to subjective effects in these studies. Therefore, a further goal of the present study was to assess associations across subjects between plasma exposure to LSD and the pharmacodynamic effects at corresponding times.

The present study combined data from two similar clinical studies that tested 100- and 200-µg doses of LSD in 24 and 16 healthy subjects, respectively. The pharmacokinetic data and concentration–effect relationship of 100 µg LSD are presented. Similar data on 200 µg LSD have been previously reported [23]. In the present study, plasma concentrations after 200 µg LSD administration were newly measured using a more sensitive and specific analytical method. The results were included for comparisons with the 100-µg data and to newly evaluate dose/concentration–response effects. The subjective effects of LSD have been reported for both doses, but relationships to plasma exposure were not evaluated [21].

2 Methods

2.1 Study Design

We performed the pharmacokinetic data analysis on two similar previously performed studies [21–23] using double-blind, placebo-controlled, cross-over designs with two experimental test sessions (LSD and placebo) in a balanced order. Study 1 used a dose of LSD 100 µg and placebo in 24 subjects. Study 2 used LSD 200 µg and placebo in 16 subjects. The washout periods between sessions were at least 7 days. The studies were registered at ClinicalTrials.gov (NCT02308969, NCT01878942).

2.2 Participants

Forty healthy participants were recruited from the University of Basel campus via an online advertisement. Twenty-four subjects [12 men, 12 women; age 33 ± 11 years (mean ± standard deviation); range

25–60 years; body weight: 68 ± 8 kg, 55–85 kg) participated in Study 1 (100 µg), and 16 subjects (eight men, eight women; age 29 ± 6 years; range 25–51 years; body weight: 72 ± 12 kg, 52–98 kg) participated in Study 2 (200 µg). The inclusion and exclusion criteria were identical for both studies. The exclusion criteria were age <25 years or >65 years, pregnancy (urine pregnancy test at screening and before each test session), personal or family (first-degree relative) history of major psychiatric disorders (assessed by the semi-structured clinical interview for *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition, Axis I disorders by the study physician and an additional interview by a trained psychiatrist), use of medications that may interfere with the study drug, chronic or acute physical illness (abnormal physical examination, electrocardiogram, or hematological and chemical blood analyses), tobacco smoking (more than ten cigarettes/day), lifetime prevalence of illicit drug use more than ten times (except for tetrahydrocannabinol), illicit drug use within the previous 2 months, and illicit drug use during the study. We performed urine drug tests at screening and before each test session, and no substances were detected during the study. The subjects were asked to abstain from excessive alcohol consumption between test sessions and particularly limit their use to one standard drink on the day before the test sessions. Additionally, the participants were not allowed to drink xanthine-containing liquids after midnight before the study day. The participants did not regularly use medications that could potentially interact with the study drug. No other medications aside from LSD were used during the study sessions. Eleven subjects had previously used a hallucinogen, including LSD (six participants), one to three times during their lives, and most of the subjects (29) were hallucinogen naive.

2.3 Study Procedures

Each study included a screening visit, a psychiatric interview, two 25-h experimental sessions, and an end-of-study visit. The experimental sessions were conducted in a quiet standard hospital patient room. The participants were resting in hospital beds except when going to the restroom. Only one research subject and one investigator were present during the experimental sessions. The participants could interact with the investigator, rest quietly, and/or listen to music via headphones, but no other entertainment was provided. LSD or placebo was administered at 9:00 A.M. A standardized lunch and dinner was served at 1:30 P.M. and 5:30 P.M., respectively. The subjects were never alone during the first 12 h after drug administration, and the investigator was in a room next to the subject for up to 24 h while the subject was asleep (mostly from 1:00 A.M. to 8:00 A.M.).

2.4 Study Drug

Lysergic acid diethylamide (*d*-lysergic acid diethylamide hydrate, high-performance liquid chromatography purity >99%; Lipomed AG, Arlesheim, Switzerland) was administered in a single oral dose of 100 or 200 µg as a capsule (Bichsel Laboratories, Interlaken, Switzerland). Both doses were within the range of doses that are taken for recreational purposes [1]. The 200-µg dose (the same capsules) was also used in LSD-assisted psychotherapy in patients [6], and intravenous doses of 75–100 µg have been used in fMRI studies in healthy subjects [13].

2.5 Measures

2.5.1 Blood Sampling

Blood was collected into lithium heparin tubes before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 16, and 24 h after LSD administration. The 0.5-, 1.5-, and 2.5-h samples were not collected in Study 1 (100 µg). The blood samples were immediately centrifuged, and the plasma was rapidly stored at -20 °C and later at -80 °C until analysis within 12 months. Long-term stability has been shown for LSD when kept under refrigerated or frozen conditions [26, 27]. Samples were thawed for the first time for both analyses, this was also the case for study 2 (200 µg) because separate sets of samples were stored and used for the present [28] and previous [29] analyses.

2.5.2 Analysis of Lysergic Acid Diethylamide Concentrations

Lysergic acid diethylamide concentrations in plasma were determined using sensitive and validated liquid chromatography-tandem mass spectrometry methods as reported in detail elsewhere [28, 29]. The lower limit of quantification was 0.05 ng/mL in Study 1 (100 µg) [29] and 0.01 ng/mL in Study 2 (200 µg) [28].

2.5.3 Subjective Mood

Visual analog scales (VASs) were repeatedly used to assess subjective effects over time [21, 22]. The VASs included separate measures for “any drug effect,” “good drug effect,” and “bad drug effect” and were presented as 100-mm horizontal lines (0–100%) marked from “not at all” on the left to “extremely” on the right. The VASs were administered 1 h before and 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 16, and 24 h after drug administration. The 0.5- and 2.5-h ratings were not collected in Study 1 (100 µg).

2.5.4 Vital Signs

Blood pressure, heart rate, and body temperature were assessed repeatedly 1 h before and 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 24 h after drug administration. Diastolic and systolic blood pressure and heart rate were measured using an automatic oscillometric device (OMRON Healthcare Europe NA, Hoofddorp, Netherlands). The measurements were performed in duplicate at an interval of 1 min and after a resting time of at least 10 min. The averages were calculated for analysis. Core (tympanic) temperature was measured using a GENIUSTM 2 ear thermometer (Tyco Healthcare Group LP, Watertown, NY, USA). The 0.5- and 2.5-h measures were not collected in Study 1 (100 µg).

2.6 Pharmacokinetic Analyses and Pharmacokinetic-Pharmacodynamic Modeling

All of the analyses were performed using Phoenix WinNonlin 6.4 (Certara, Princeton, NJ, USA). Pharmacokinetic parameters were estimated using compartmental modeling. A one-compartment model was used with first-order input, first-order elimination, and no lag time. Initial estimates for apparent volume of distribution and λ were derived from non-compartmental analyses.

The model fit was not relevantly improved by a two-compartment model based on visual inspection of the plots. The one-compartment model showed better Akaike information criterion values in all subjects than a two-compartment model. The pharmacokinetic model was first fitted and evaluated. The predicted concentrations were then used as inputs to the pharmacodynamic model, treating the pharmacokinetic parameters as fixed and using the classic pharmacokinetic/pharmacodynamic link model module in WinNonlin. The model uses a first-order equilibrium rate constant (k_{eo}) that related the observed pharmacodynamic effects of LSD to the estimated LSD concentrations at the effect site (Fig. S1) and accounts for the lag between the plasma- and effect-site concentration curves [30]. Initial estimates for k_{eo} values were obtained using semi-compartmental modeling by collapsing the hysteresis loop in the C_e vs. effect plots in WinNonlin. A sigmoid maximum effect (E_{max}) model (EC_{50} , E_{max} , γ) was selected for all pharmacodynamic effects. EC_{50} and E_{max} estimates were taken from the pharmacokinetic-pharmacodynamic plots. Lower and upper limits for E_{max} were set to 0 and 100%, respectively, for all the VAS scores. Upper limits for E_{max} for changes in heart rate, body temperature, and diastolic and systolic blood pressure were set to 100/min, 2 °C, 50 and 80 mm Hg, respectively. The sigmoidal E_{max} model best described the relationship between

estimated effect-site concentrations and the effects of LSD compared with a simple E_{max} model (plot inspection and Akaike information criteria). Examples of diagnostic plots are shown in Figs. S8 and S9.

2.7 Statistical Analyses

The LSD-induced subjective and autonomic effects were determined as a difference from placebo in the same subject at the corresponding time point to control for circadian changes and placebo effects [22]. The pharmacodynamic effect changes after LSD administration for each time point were plotted over time (effect-time curves) and against the respective plasma concentrations of LSD and graphed as concentration-effect curves. The onset, time to maximum plasma concentration (T_{max}), offset, and effect duration were assessed for the model-predicted “any drug effect” VAS effect-time plots after LSD using a threshold of 10% of the maximal possible effect of 100% using Phoenix WinNonlin 6.4. Associations between concentrations and effects were assessed using Pearson correlations, and multiple regression analysis was used to exclude effects of sex and body weight (Statistica 12 software; StatSoft, Tulsa, OK, USA).

3 Results

3.1 Pharmacokinetics

The plasma concentration-time curves for the two LSD doses are shown in Fig. 1a. The pharmacokinetic parameters are shown in Table 1. In Study 1 (100 µg), LSD could be quantified up to 8, 10, 12, 16, and 24 h in 24, 23, 22, 9, and one subject, respectively. In Study 2 (200 µg), LSD could be quantified up to 16 h in all 16 subjects and up to

Fig. 1 Pharmacokinetics and pharmacodynamics of lysergic acid diethylamide (LSD). **a** LSD plasma concentration-time curves. The corresponding semi-log plot is shown in Fig. S3. LSD effect-time curves for Visual Analog Scale ratings (0–100%) of **b** “any drug effect,” **d** “good drug effect,” and **f** “bad drug effect.” **c**, **e**, **g** In the LSD concentration-effect plots (hysteresis curves), the subjective effects of LSD showed moderate counterclockwise hysteresis, indicating a relatively short delay in the effect of LSD relative to the changes in plasma concentration over time. The plasma concentration-effect site equilibration half-lives were in the range of 21–48 min according to the pharmacokinetic-pharmacodynamic link model (Table 2). “Any drug effect” and “good drug effect” were robustly and markedly increased in all subjects and paralleled the changes in LSD concentration, whereas the mean “bad drug effect” increased only moderately after LSD owing to transient increases. “Bad drug effect” occurred mostly at the onset of the drug effect in some subjects but also later in time in others. The data are expressed as the mean \pm standard error of the mean in 24 and 16 subjects after administration of 100 and 200 µg LSD, respectively. The time of sampling is noted next to each point. LSD was administered at $t = 0$

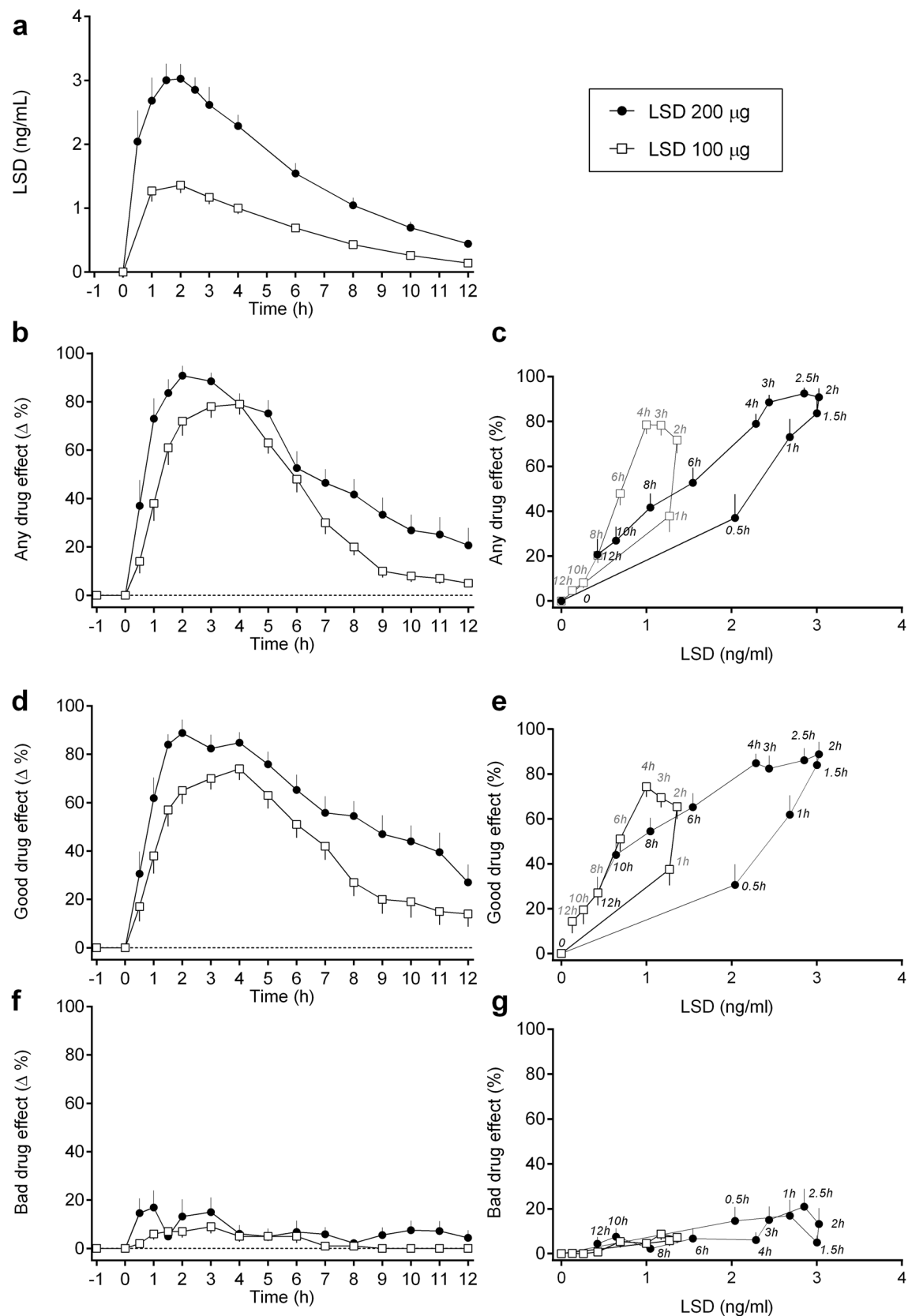


Table 1 Pharmacokinetic parameters for LSD based on compartmental modeling

Dose	<i>N</i>		<i>k</i> ₀₁ (1/h)	λ (1/h)	<i>V</i> _d (L)	<i>C</i> _{max} (ng/mL)	<i>t</i> _{max} (h)	<i>t</i> _{1/2} (h)	AUC _∞ (ng·h/mL)	CL/ <i>F</i> (L/h)
100 µg	24	Geometric mean	1.4	0.27	46	1.3	1.4	2.6	8.1	12.3
		(95% CI)	(1.2–4.1)	(0.24–0.31)	(35–76)	(1.2–1.9)	(1.3–2.1)	(2.4–3.0)	(7.5–11.1)	(7.8–24)
		Range	0.31–9.9	0.17–0.50	24–270	0.3–3.7	0.4–3.2	1.4–4.2	1–19	5.2–103
200 µg	16	Geometric mean	1.2	0.27	37	3.1	1.5	2.6	20.3	9.9
		(95% CI)	(0.68–4.6)	(0.22–0.35)	(32–46)	(2.6–4.0)	(1.3–2.4)	(2.2–3.4)	(17.3–26.2)	(8.3–12.8)
		Range	0.27–10	0.12–0.59	18–66	1.9–7.1	0.4–3.8	1.2–5.6	11–39	5.1–18.5

AUC_∞ area under the plasma concentration-time curve from time zero to infinity, *C*_{max} estimated maximum plasma concentration, *t*_{1/2} estimated plasma elimination half-life, *t*_{max} estimated time to reach *C*_{max}, *k*₀₁ first-order absorption coefficient, λ first order elimination coefficient, *V*_d volume of distribution

24 h in 15 subjects (Fig. S2). Mean maximum plasma concentration (*C*_{max}) and area under the concentration-time curve values were approximately twice as high for the 200-µg dose compared with the 100-µg dose. Dose-normalized *C*_{max} and area under the concentration-time curve values were not statistically different between the dose groups and the *T*_{max} and plasma half-lives were also similar, consistent with dose-proportional pharmacokinetics (Table 1). Consistent with the fit of the one-compartment model, inspection of the semi-logarithmic concentration-time curves showed linear elimination kinetics for both doses (Fig. S3) up to 12 h as previously reported for the 200-µg dose [23]. The individual-observed and model-predicted LSD concentrations are shown in Fig. S2. Plasma concentrations varied considerably between subjects, especially at the lower 100-µg dose (Table 1; Fig. S2).

3.2 Pharmacodynamics

Lysergic acid diethylamide produced robust increases in “any drug effect” (Fig. 1b, Fig. S4) and “good drug effect” (Fig. 1d, Fig. S5). Transient “bad drug effect” was reported in some subjects, resulting in a moderate increase in mean group ratings (Fig. 1f, Fig. S6). The corresponding subjective peak effects have previously been reported and were shown to be dose dependent [21]. “Any drug effect,” “good drug effect,” and “bad drug effect” ratings for each subject are shown in Figs. S4–6, respectively. After administration of the 100-µg dose of LSD, the times of onset and offset of the subjective response, assessed by the “any drug effect” VAS, were (mean ± standard deviation) 0.8 ± 0.4 h (range 0.1–1.7 h) and 9.0 ± 2.0 h (range 6.1–14.5 h), respectively. The mean effect duration was 8.2 ± 2.1 h (range 5–14 h). The time to peak drug effect was 2.8 ± 0.8 h (range 1.2–4.6 h). After administration of the 200-µg dose of LSD, the times of onset and offset of the subjective response were 0.4 ± 0.3 h (range 0.04–1.2 h) and 11.6 ± 4.2 h (range 7.0–19.5 h), respectively. The mean effect duration was 11.2 ± 4.2 h (range 6.4–19.3 h). The time to the subjective peak response was

2.5 ± 1.2 h (range 0.8–4.4 h). LSD increased diastolic and systolic blood pressure, heart rate, and body temperature compared with placebo to similar extents for both doses (Fig. 2). The corresponding peak effect data and dose-response statistics have been previously reported [21].

3.3 Pharmacokinetic-Pharmacodynamic Modeling

Figures 1 and 2 show the subjective, cardiovascular, and thermogenic effects of LSD plotted against the plasma concentration over time. A close relationship was found between LSD concentrations and LSD effects over time. Counterclockwise hysteresis was observed during the assumed drug distribution phase (<2 h), especially for body temperature (Fig. 2h). Model-predicted effects of LSD on the VASs for “any drug effect,” “good drug effect,” and “bad drug effect” are illustrated for each subject in Figs. S4–6, respectively. Table 2 shows the predicted concentrations of LSD at the effect site that produced half-maximal effects (EC₅₀ values). Mean EC₅₀

Fig. 2 Pharmacokinetics and autonomic effects in response to lysergic acid diethylamide (LSD). The figure shows LSD effect-time curves for **a** diastolic blood pressure, **c** systolic blood pressure, **e** heart rate, and **g** changes in body temperature and corresponding **b**, **d**, **f**, **h** LSD concentration-effect plots (hysteresis curves). The cardiovascular stimulant effects of LSD at the higher 200-µg dose showed only little counterclockwise hysteresis, indicating a short delay in the effect of LSD relative to the changes in plasma concentration over time and thus a close relationship between LSD concentration and changes in cardiovascular effects over time within subjects. The plasma concentration-effect site equilibration half-lives were in the range of 13–34 min according to the pharmacokinetic-pharmacodynamic link model (Table 2). In contrast, marked counterclockwise hysteresis was observed in the LSD concentration-body temperature change plot, indicating that the LSD-induced changes in body temperature manifested themselves slowly and with a mean plasma concentration-effect site equilibration half-life of 136 min for the 200-µg dose (Table 2). The data are expressed as the mean ± standard error of the mean in 24 and 16 subjects after administration of LSD 100 and 200 µg, respectively. The pharmacodynamic values are the mean ± standard error of the mean differences from placebo at each time point. The time of sampling is noted next to each point. LSD was administered at *t* = 0

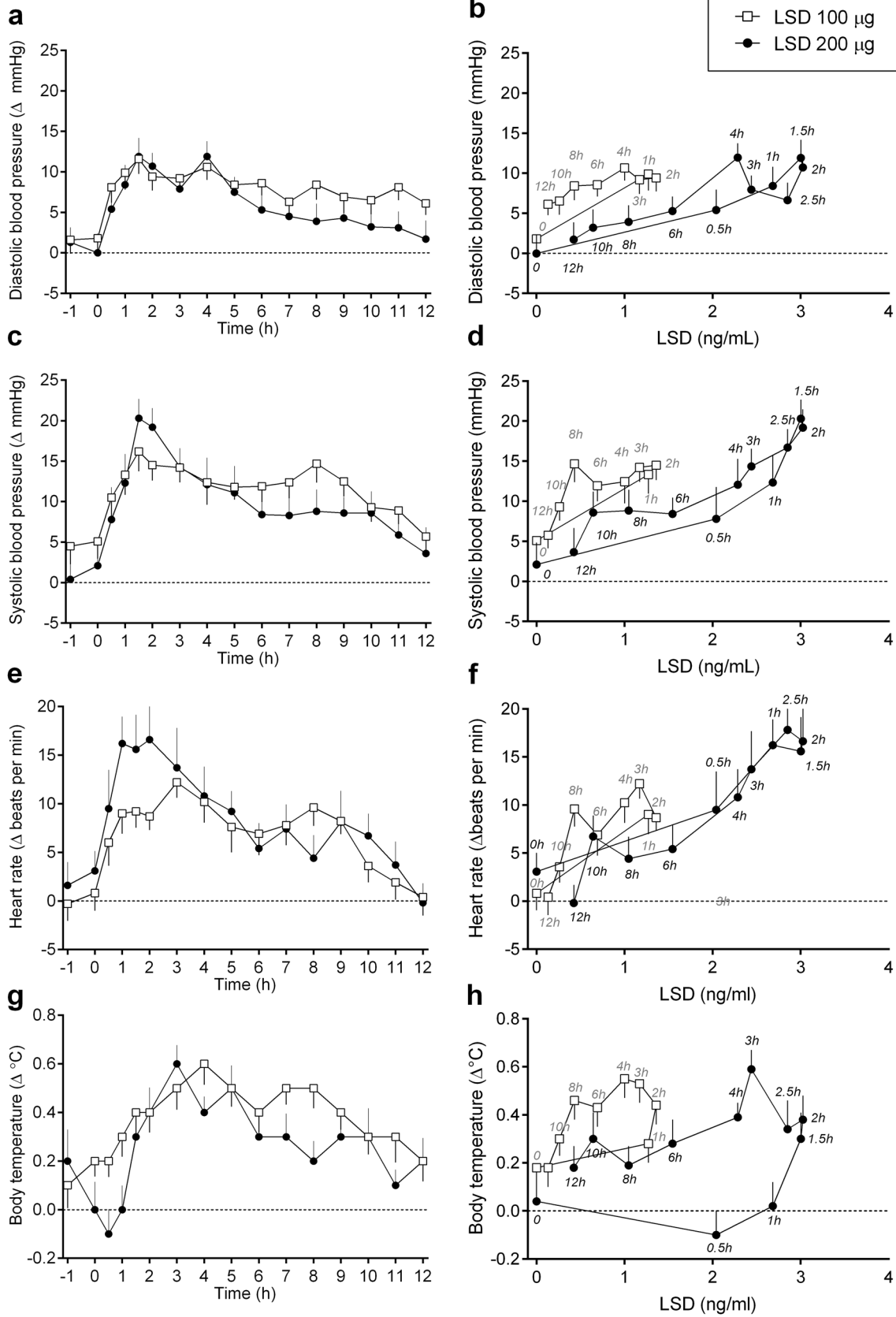


Table 2 Pharmacodynamic parameter estimates (PK-PD link model)

Effect	Dose	EC ₅₀ (ng/mL)	E _{max} (%)	γ	k _{eo} (1/h)	T _{1/2} k _{eo} (min)
Any drug effect	100 µg	0.75 ± 0.4	95 ± 9	4.2 ± 1.2	1.8 ± 1.3	35 ± 23
	200 µg	1.2 ± 0.7	97 ± 5	3.4 ± 1.5	3.4 ± 1.7	21 ± 17
Good drug effect	100 µg	0.71 ± 0.5	89 ± 15	3.9 ± 1.5	2.0 ± 1.6	39 ± 37
	200 µg	0.94 ± 0.5	93 ± 9	3.2 ± 1.6	2.4 ± 1.8	32 ± 29
Bad drug effect	100 µg	1.5 ± 1.1	32 ± 37	4.7 ± 2.3	2.7 ± 2.2	42 ± 37
	200 µg	2.5 ± 1.6	34 ± 35	3.2 ± 2.1	2.8 ± 2.0	48 ± 66
Heart rate increase	100 µg	0.67 ± 0.5	22 ± 25	3.7 ± 2.0	2.5 ± 1.9	46 ± 52
	200 µg	1.9 ± 1.2	33 ± 28	2.7 ± 1.8	4.0 ± 2.0	13 ± 8
Body temperature increase	100 µg	0.75 ± 0.4	1.1 ± 0.6	2.2 ± 1.8	1.5 ± 1.6	107 ± 121
	200 µg	1.8 ± 1.1	1.0 ± 0.6	3.6 ± 2.0	1.7 ± 1.9	136 ± 155
Diastolic blood pressure increase	100 µg	0.9 ± 0.6	23 ± 14	2.0 ± 1.6	2.6 ± 1.9	53 ± 70
	200 µg	1.6 ± 0.9	18 ± 11	3.5 ± 1.6	3.4 ± 1.9	31 ± 42
Systolic blood pressure increase	100 µg	0.8 ± 0.5	30 ± 17	1.9 ± 1.6	2.6 ± 1.7	51 ± 78
	200 µg	1.9 ± 1.4	30 ± 17	2.9 ± 1.9	3.2 ± 1.9	34 ± 41

Values are means ± standard deviations. $T_{1/2}k_{eo} = \ln 2/k_{eo}$, calculated for each individual value

EC₅₀ maximal effect predicted by the PK-PD link model, EC₅₀ predicted drug concentration at effect site producing a half-maximal effect, γ sigmoid shape parameter, k_{eo} first-order rate constant for the equilibration process between plasma concentration and effect site (PK-PD model link parameter), $t_{1/2}k_{eo}$ (min) plasma-effect-site equilibration half-life

values were in the range of 0.67–2.5 ng/mL and lower for “good drug effect” than for “bad drug effect” (Table 2). “Any drug effect” and “good drug effect” could be modeled in all of the subjects, whereas no “bad drug effect” (ratings <5% at any time point) was reported in eight (33%) and five (31%) subjects after 100 and 200 µg, respectively. Thus, the EC₅₀ and k_{eo} values could not be determined in these subjects. Similarly, vital signs did not change sufficiently in a few subjects (one to three/outcome) to determine these values.

The predicted C_{max} of LSD did not correlate with the predicted maximal response on the “any drug effect” VAS when analyzed across subjects and separately for the two dose groups ($R_p = 0.38$, $p = 0.08$, and $R_p = 0$, $p = 0.9$, for the 100- and 200-µg doses, respectively). There was a significant correlation in the pooled sample ($R_p = 0.38$, $p < 0.05$, $n = 40$, Fig. S7). The predicted area under the concentration-time curve of LSD did not correlate with the predicted area under the concentration-time curve for “any drug effect”, a measure of the overall pharmacodynamic response ($R_p = 0$, $p = 0.9$, and $R_p = 0.27$, $p = 0.4$, respectively). Additionally, there were generally no correlations between plasma LSD concentrations and different pharmacodynamic effects for matched time points across subjects within dose groups (Table 3). A few correlations were significant at the beginning (1 h) and end (8 and 12 h) of the LSD effect. However, no significant associations were found between plasma concentrations and effects during the peak response to LSD (3–6 h). Multiple regression analysis, including LSD concentration, body weight, and sex, revealed no associations between the

effects of LSD and any of these possible predictors. Thus, the plasma concentrations of LSD did not predict the effects of LSD during the time it produced robust and similar effects in all of the subjects (i.e., little between-subject variability). In contrast, a close relationship was found over time within subjects, as shown in the pharmacokinetic-pharmacodynamic analysis (Figs. 1, 2).

4 Discussion

The present study describes the pharmacokinetics and concentration–effect relationship after oral administration of LSD 100 µg. Additionally, the previously reported pharmacokinetics and concentration–effect relationship for the 200-µg dose of LSD [23] were reanalyzed and included for comparison with the 100-µg dose. Compartmental modeling predicted geometric mean peak plasma concentrations of 1.3 ng/mL, 1.4 h after administration of the 100-µg dose. Mean C_{max} values of 3.1 ng/mL were reached after 1.5 h after administration of the 200-µg dose. The predicted mean half-lives of LSD were 2.6 h after both doses. The plasma half-life in the present study was comparable to the value of 2.9 h after intravenous administration of 2 µg/kg of LSD [24] but shorter than the 3.6-h value previously determined using non-compartmental analysis [23]. Additionally, the plasma concentrations after administration of the 200-µg dose in the present study were lower than those that were previously published in the same research subjects [23]. This can be explained by the different analytical methods and modeling approach that were

Table 3 Correlations between plasma levels of LSD and its pharmacodynamic effects at the corresponding time points after administration

Effect			1 h	2 h	3 h	4 h	6 h	8 h	10 h	12 h
Any subjective drug effect	100 µg	<i>N</i> = 24	0.17	0.13	−0.02	−0.04	−0.18	0.09	0.01	−0.03
	200 µg	<i>N</i> = 16	0.21	0.17	0.1	0.13	0.2	0.16	0.33	0.42
	Both	<i>N</i> = 40	0.36	0.35	0.19	0.04	0.06	0.41	0.46	0.49
Good drug effect	100 µg	<i>N</i> = 24	0.6	0.3	0.23	0.15	−0.13	−0.2	−0.03	0.04
	200 µg	<i>N</i> = 16	0	−0.23	0.32	0.27	0.28	0.55	0.39	0.17
	Both	<i>N</i> = 40	0.39	0.34	0.36	0.31	0.24	0.42	0.35	0.23
Bad drug effect	100 µg	<i>N</i> = 24	0.06	−0.11	−0.23	−0.1	−0.08	−0.03	0	−0.15
	200 µg	<i>N</i> = 16	0.34	−0.32	−0.27	0.07	0.2	0.35	−0.26	−0.16
	Both	<i>N</i> = 40	0.36	−0.16	0	0	0.1	0.29	0.05	0.07
Heart rate increase	100 µg	<i>N</i> = 24	0.41	0.3	0.4	0.27	0.1	0.26	−0.4	0.027
	200 µg	<i>N</i> = 16	0.3	0.21	0.3	−0.06	−0.08	0.19	−0.16	−0.52
	Both	<i>N</i> = 40	0.44	0.41	0.33	0.08	−0.05	−0.02	0.03	−0.2
Body temperature increase	100 µg	<i>N</i> = 24	0.12	−0.27	0.14	0.07	0.18	−0.06	−0.2	0.41
	200 µg	<i>N</i> = 16	0.09	−0.11	0.54	−0.1	−0.02	0.37	0.15	−0.19
	Both	<i>N</i> = 40	−0.08	−0.18	0.25	−0.15	−0.09	−0.12	0.02	0.06
Diastolic blood pressure increase	100 µg	<i>N</i> = 24	0.16	−0.09	0.14	0.04	0.17	0.15	0.28	0.13
	200 µg	<i>N</i> = 16	−0.53	−0.22	0.2	−0.13	0.09	0.27	0.09	0.47
	Both	<i>N</i> = 40	−0.2	−0.03	0.07	0.03	−0.06	−0.01	0.01	0.07
Systolic blood pressure increase	100 µg	<i>N</i> = 24	0.1	0.05	0.06	0	0.2	0.23	0.29	0.21
	200 µg	<i>N</i> = 16	−0.03	−0.4	−0.1	0.25	0	0.54	−0.02	0.19
	Both	<i>N</i> = 40	0	0.07	0.03	0.07	−0.07	0.11	0.05	0.08

Data are Pearson correlation coefficients between the LSD concentration in plasma and the corresponding time-matched effect of LSD. Bold values indicate significant associations ($p < 0.05$)

used in the present study, which predicts lower C_{\max} values than the observed values. Overall, we observed linear dose and elimination kinetics of LSD up to 12 h after drug administration.

The present data on the plasma concentration-time curves of LSD are important because many experimental and therapeutic studies are currently being conducted or have been published without this detailed information on the presence of LSD in the human body. Specifically, the effects of LSD on emotion processing after 100 and 200 µg have been reported [23], but no pharmacokinetic data were reported. Additionally, fMRI data were obtained in Study 1 (100 µg) in Basel and in an additional study in Zurich ($n = 22$) that did not perform blood sampling. Doses of 100 µg were used in both studies. Thus, the present study provides estimates of LSD concentrations in plasma over time for these studies and the observed and predicted time courses of the subjective and autonomic effects of LSD. The 200-µg dose preparation of LSD has been used in patients [5, 6], and the present phase I study provides the pharmacokinetic data for these phase II studies.

In contrast, no data are currently available on the plasma concentrations of LSD after intravenous administration of 75 µg of LSD base in saline [11], despite the publication of extensive pharmacodynamic data using this preparation

and route of administration [10–19]. The intravenous 75-µg dose of LSD produced comparably strong alterations in consciousness to the 100-µg dose in the present study [10, 31]. Additionally, the time-concentration curve for the 75-µg intravenous preparation remains unknown. Specifically, an intravenous bolus dose of LSD would be expected to result in peak effects shortly after administration. Indeed, early studies reported that intravenous administration of LSD tartrate salt at a higher dose (2 µg/kg of base) produced a rapid onset within seconds to minutes and peak effects that occurred approximately 30 min after administration [24, 32–34].

In the more recent studies that used the 75-µg dose administered as the base, subjective drug effects reportedly began within 5–15 min and peaked 45–90 min after intravenous dosing, although further details were not reported [13, 19]. Other hallucinogens with mechanisms of action that are similar to those of LSD (e.g., serotonin 5-HT_{2A} receptor stimulation [35]), such as dimethyl-tryptamine or psilocybin, also produced subjective and autonomic effects almost instantaneously and peak effects within 2–5 min after intravenous administration [36–38]. In the present study, the mean effect onset and peak were 48 and 170 min, respectively, after oral administration of LSD 100 µg. Thus, the effect began and peaked an average

of 30 and 100 min later, respectively, after oral administration compared with intravenous administration of an equivalent dose [13, 19]. Magnetic resonance imaging scanning correctly started at approximately 70 and 150 min in the studies that used intravenous [13] and oral (unpublished data from Study 1, 100 µg) routes of LSD administration, respectively, coinciding with the maximal response to LSD. Nevertheless, the plasma concentrations of LSD and associated time-matched subjective responses after intravenous LSD administration should also be determined to better evaluate the considerable research data that have been generated with this formulation.

After intravenous administration, a drug is rapidly diluted and distributed within the blood. Peak plasma concentrations are typically reached rapidly, and elimination begins immediately. Using the model parameters λ and k_{eo} from the present study, the T_{max} for “any drug effect” after intravenous administration can be predicted to occur at approximately 70 and 50 min for the 100- and 200-µg doses and are thus similar to the recently observed times to peak effects [13, 19]. In our model, the relatively long T_{max} of the effect of LSD is represented by the lag that is attributable to distribution of the drug from plasma to the hypothetical effect compartment. The cause for this lag is unclear. Additional studies are needed to determine whether LSD is distributed slowly because it is present only in small concentrations or slowly penetrates the blood–brain barrier or whether there is a lag in the response mechanism.

The present study showed that LSD produced robust and high subjective “any drug effect” and “good drug effect” in almost all of the subjects. The estimates of the corresponding EC_{50} values were in the range of 0.71–1.2 ng/mL and lower than the mean LSD C_{max} values (1.3 and 3.1 ng/mL for the 100- and 200-µg doses, respectively) observed in the present study. “Bad drug effects” were moderate and not present in every subject. Consistent with this finding, the EC_{50} values were higher than those for “good drug effect” and “any drug effect” (1.5–2.5 ng/mL). As previously reported, the subjective effects were dose dependent, whereas the autonomic effects were comparable at both doses [21]. When analyzed within subjects using pharmacokinetic–pharmacodynamic modeling, a close relationship was found between plasma concentrations of LSD and the effects of LSD, with moderate counterclockwise hysteresis. Counterclockwise hysteresis typically reflects the time lag that is caused by drug distribution to the effect site and the response time associated with the mechanism of action. The present study showed that the subjective and autonomic effects establish themselves relatively slowly. On average, the subjective “any drug effect” peak was reached 2.8 and 2.5 h after administration of the 100- and 200-µg doses, respectively, and 1.1 and 0.6 h after the respective peak LSD concentrations were reached. The lag times were

comparable for the increases in heart rate and blood pressure but longer for the thermogenic response. No clockwise hysteresis was found for any of the pharmacodynamic outcome measures, and thus no evidence was found of acute tolerance as described for other psychoactive substances, such as methylenedioxymethamphetamine [39] or cocaine [40], or for repeated administration of LSD [41]. Thus, as long as relevant concentrations of LSD were present in plasma, subjective and autonomic effects were observed. The mean durations of the subjective effects of LSD was 8 and 11 h after administration of the 100- and 200-µg doses, respectively, and the difference corresponded to the plasma half-life of LSD.

The present analyses typically found no correlations between LSD concentrations and the effects of LSD across subjects within dose groups, likely because of the relatively high concentrations of LSD and generally consistently high subjective response ratings in most subjects. If relatively high and similar doses of LSD are used that result in plasma concentrations above the EC_{50} of a particular response measures, then responses do not vary across subjects because responses are close to maximal. This would typically also be the case with measures with a maximal effect limit such as VAS ratings and some physiological effects such as pupil size [42]. In fact, responses to LSD or other drugs in a standardized experimental setting may vary only if the response is not induced consistently in all subjects (e.g., at the beginning and end of the response) because of individual differences in drug absorption/distribution and elimination. Correlations of plasma concentrations with the subjective and cardiovascular effects of LSD or 3,4-methylenedioxymethamphetamine [42] across subjects are only weak during the peak response. This finding needs to be considered when interpreting associations between subjective responses and other measures, such as fMRI parameters. fMRI findings may reflect the variance in LSD plasma concentrations. The likelihood of detecting correlations within a dose group increases for effects that are not robustly induced in all subjects.

The present study has limitations. First, the two doses of LSD were evaluated in two separate studies in different participants and not within subjects. Second, the plasma samples were analyzed in different laboratories. Nonetheless, the pharmacokinetic data were consistent across the two studies and laboratories.

5 Conclusion

We gathered pharmacokinetic data for oral LSD that are essential for interpreting the findings of clinical studies and LSD intoxication. LSD had dose-proportional pharmacokinetics and first-order elimination up to 12 h. A close

plasma concentration–effect relationship was found within subjects over time, with moderate counterclockwise hysteresis because of a short lag of the response. Generally, no association was found between plasma LSD concentrations and its robust effects when analyzed across different subjects and within a dose group. This has implications for studies that interrelate different effects of LSD.

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Author contributions PD designed the research, performed the research, and analyzed the data. YS designed the research and performed the research. AES performed the research and analyzed the data. TK, FH and KMR analyzed the data. MEL designed the research, analyzed the data, and wrote the manuscript.

Compliance with Ethical Standards

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Conflict of interest Patrick C. Dolder, Yasmin Schmid, Andrea E. Steuer, Thomas Kraemer, Katharina M. Rentsch, Felix Hammann, and Matthias E. Liechti declare no conflicts of interest.

Ethics approval and consent to participate The studies were conducted in accordance with the Declaration of Helsinki and approved by the local ethics committee. The administration of LSD to healthy subjects was authorized by the Swiss Federal Office for Public Health, Bern, Switzerland. All of the subjects provided written consent before participating in either of the studies, and they were paid for their participation.

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References

1. Passie T, Halpern JH, Stichtenoth DO, et al. The pharmacology of lysergic acid diethylamide: a review. *CNS Neurosci Ther.* 2008;14(4):295–314.
2. Nichols DE. Psychedelics. *Pharmacol Rev.* 2016;68(2):264–355.
3. Krebs TS, Johansen PO. Over 30 million psychedelic users in the United States. *F1000Res.* 2013;2:98.
4. Davenport WJ. Psychedelic and nonpsychedelic LSD and psilocybin for cluster headache. *CMAJ.* 2016;188(3):217.
5. Gasser P, Kirchner K, Passie T. LSD-assisted psychotherapy for anxiety associated with a life-threatening disease: a qualitative study of acute and sustained subjective effects. *J Psychopharmacol.* 2015;29(1):57–68.
6. Gasser P, Holstein D, Michel Y, et al. Safety and efficacy of lysergic acid diethylamide-assisted psychotherapy for anxiety associated with life-threatening diseases. *J Nerv Ment Dis.* 2014;202(7):513–20.
7. Baumeister D, Barnes G, Giaroli G, Tracy D. Classical hallucinogens as antidepressants? A review of pharmacodynamics and putative clinical roles. *Ther Adv Psychopharmacol.* 2014;4(4):156–69.
8. Krebs TS, Johansen PO. Lysergic acid diethylamide (LSD) for alcoholism: meta-analysis of randomized controlled trials. *J Psychopharmacol.* 2012;26(7):994–1002.
9. Kupferschmidt K. High hopes. *Science.* 2014;345(6192):18–23.
10. Carhart-Harris RL, Kaelen M, Bolstridge M, et al. The paradoxical psychological effects of lysergic acid diethylamide (LSD). *Psychol Med.* 2016;46:1379–90.
11. Tagliazucchi E, Roseman L, Kaelen M, et al. Increased global functional connectivity correlates with LSD-induced ego dissolution. *Curr Biol.* 2016;26(8):1043–50.
12. Kaelen M, Roseman L, Kahan J, et al. LSD modulates music-induced imagery via changes in parahippocampal connectivity. *Eur Neuropsychopharmacol.* 2016;26:1099–109.
13. Carhart-Harris RL, Muthukumaraswamy S, Roseman L, et al. Neural correlates of the LSD experience revealed by multimodal neuroimaging. *Proc Natl Acad Sci.* 2016;113:4853–8.
14. Terhune DB, Luke DP, Kaelen M, et al. A placebo-controlled investigation of synaesthesia-like experiences under LSD. *Neuropsychologia.* 2016;88:28–34.
15. Speth J, Speth C, Kaelen M, et al. Decreased mental time travel to the past correlates with default-mode network disintegration under lysergic acid diethylamide. *J Psychopharmacol.* 2016;30(4):344–53.
16. Roseman L, Sereno MI, Leech R, et al. LSD alters eyes-closed functional connectivity within the early visual cortex in a retinotopic fashion. *Hum Brain Mapp.* 2016;37:3031–40.
17. Lebedev AV, Kaelen M, Lovden M, et al. LSD-induced entropic brain activity predicts subsequent personality change. *Hum Brain Mapp.* 2016;37:3203–13.
18. Carhart-Harris RL, Kaelen M, Whalley MG, et al. LSD enhances suggestibility in healthy volunteers. *Psychopharmacology.* 2015;232(4):785–94.
19. Kaelen M, Barrett FS, Roseman L, et al. LSD enhances the emotional response to music. *Psychopharmacology.* 2015;232(19):3607–14.
20. Strajhar P, Schmid Y, Liakoni E, et al. Acute effects of lysergic acid diethylamide on circulating steroid levels in healthy subjects. *J Neuroendocrinol.* 2016;28:12374.
21. Dolder PC, Schmid Y, Mueller F, et al. LSD acutely impairs fear recognition and enhances emotional empathy and sociality. *Neuropsychopharmacology.* 2016;41:2638–46.
22. Schmid Y, Enzler F, Gasser P, et al. Acute effects of lysergic acid diethylamide in healthy subjects. *Biol Psychiatry.* 2015;78(8):544–53.
23. Dolder PC, Schmid Y, Haschke M, et al. Pharmacokinetics and concentration-effect relationship of oral LSD in humans. *Int J Neuropsychopharmacol.* 2015;19:pii: pyv072.
24. Aghajanian GK, Bing OH. Persistence of lysergic acid diethylamide in the plasma of human subjects. *Clin Pharmacol Ther.* 1964;5:611–4.
25. Upshall DG, Wailling DG. The determination of LSD in human plasma following oral administration. *Clin Chim Acta.* 1972;36(1):67–73.
26. Klette KL, Horn CK, Stout PR, Anderson CJ. LC-MS analysis of human urine specimens for 2-oxo-3-hydroxy LSD: method validation for potential interferants and stability study of 2-oxo-3-hydroxy LSD under various storage conditions. *J Anal Toxicol.* 2002;26(4):193–200.
27. Martin R, Schurenkamp J, Gasse A, et al. Determination of psilocin, bufotenine, LSD and its metabolites in serum, plasma and

- urine by SPE-LC-MS/MS. *Int J Legal Med.* 2013;127(3):593–601.
28. Steuer AE, Poetzsch M, Stock L, et al. Development and validation of an ultra-fast and sensitive microflow liquid chromatography-tandem mass spectrometry (MFLC-MS/MS) method for quantification of LSD and its metabolites in plasma and application to a controlled LSD administration study in humans. *Drug Test Anal.* 2016. doi:[10.1002/dta.2042](https://doi.org/10.1002/dta.2042) (**Epub ahead of print**).
 29. Dolder PC, Liechti ME, Rentsch KM. Development and validation of a rapid turboflow LC-MS/MS method for the quantification of LSD and 2-oxo-3-hydroxy LSD in serum and urine samples of emergency toxicological cases. *Anal Bioanal Chem.* 2015;407:1577–84.
 30. Sheiner LB, Stanski DR, Vozeh S, et al. Simultaneous modeling of pharmacokinetics and pharmacodynamics: application to d-tubocurarine. *Clin Pharmacol Ther.* 1979;25(3):358–71.
 31. Liechti ME, Dolder PC, Schmid Y. Alterations in consciousness and mystical-type experiences after acute LSD in humans. *Psychopharmacology (Berl.)*. 2016;. doi:[10.1007/s00213-016-4453-0](https://doi.org/10.1007/s00213-016-4453-0) (**Epub ahead of print**).
 32. Sokoloff L, Perlin S, Kornetsky C, Kety SS. The effects of D-lysergic acid diethylamide on cerebral circulation and over-all metabolism. *Ann NY Acad Sci.* 1957;66(3):468–77.
 33. Wagner JG, Aghajanian GK, Bing OH. Correlation of performance test scores with “tissue concentration” of lysergic acid diethylamide in human subjects. *Clin Pharmacol Ther.* 1968;9(5):635–8.
 34. Hoch PH. Studies in routes of administration and counteracting drugs: lysergic acid diethylamide and mescaline in experimental psychosis. New York: Grune and Stratton; 1956.
 35. Rickli A, Moning OD, Hoener MC, Liechti ME. Receptor interaction profiles of novel psychoactive tryptamines compared with classic hallucinogens. *Eur Neuropsychopharmacol.* 2016;26:1327–37.
 36. Gallimore AR, Strassman RJ. A model for the application of target-controlled intravenous infusion for prolonged immersive DMT psychedelic experience. *Front Pharmacol.* 2016;14(7):211. doi:[10.3389/fphar.2016.00211](https://doi.org/10.3389/fphar.2016.00211).
 37. Strassman RJ, Qualls CR, Uhlenhuth EH, Kellner R. Dose-response study of *N, N*-dimethyltryptamine in humans: II. Subjective effects and preliminary results of a new rating scale. *Arch Gen Psychiatry.* 1994;51(2):98–108.
 38. Carhart-Harris RL, Williams TM, Sessa B, et al. The administration of psilocybin to healthy, hallucinogen-experienced volunteers in a mock-functional magnetic resonance imaging environment: a preliminary investigation of tolerability. *J Psychopharmacol.* 2011;25(11):1562–7.
 39. Hysek CM, Simmler LD, Ineichen M, et al. The norepinephrine transporter inhibitor reboxetine reduces stimulant effects of MDMA (“ecstasy”) in humans. *Clin Pharmacol Ther.* 2011;90(2):246–55.
 40. Van Dyke C, Ungerer J, Jatlow P, et al. Intranasal cocaine: dose relationships of psychological effects and plasma levels. *Int J Psychiatry Med.* 1982;12(1):1–13.
 41. Belleville RE, Fraser HF, Isbell H, et al. Studies on lysergic acid diethylamide (LSD-25): I. Effects in former morphine addicts and development of tolerance during chronic intoxication. *AMA. Arch Neurol Psychiatry.* 1956;76(5):468–78.
 42. Hysek CM, Liechti ME. Effects of MDMA alone and after pretreatment with reboxetine, duloxetine, clonidine, carvedilol, and doxazosin on pupillary light reflex. *Psychopharmacology.* 2012;224:363–76.

Supplementary Figures

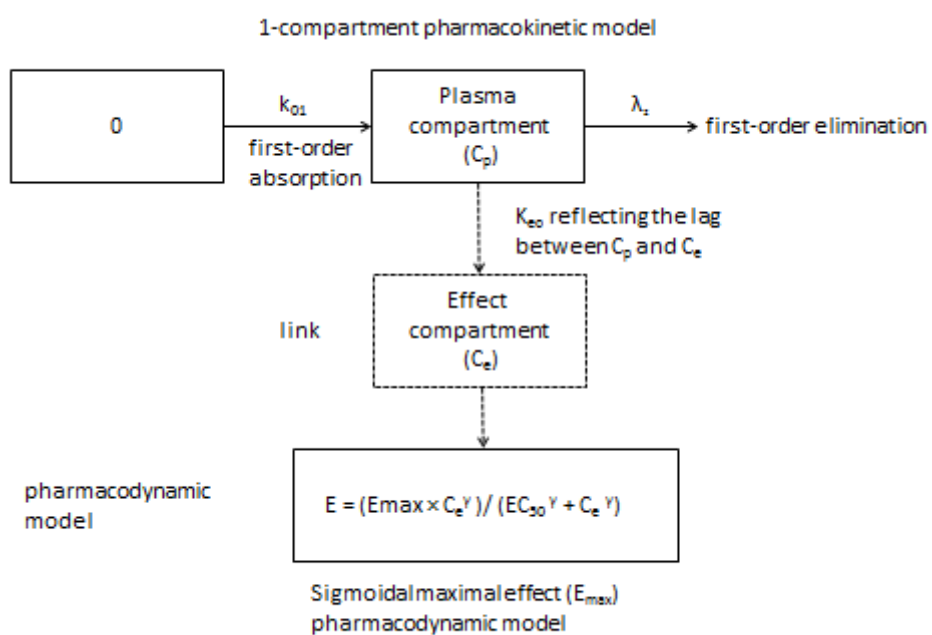
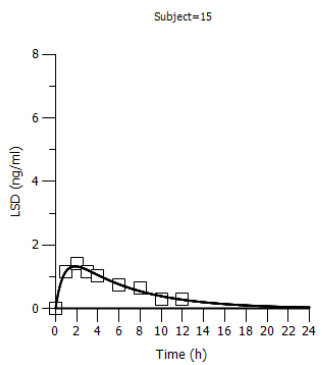
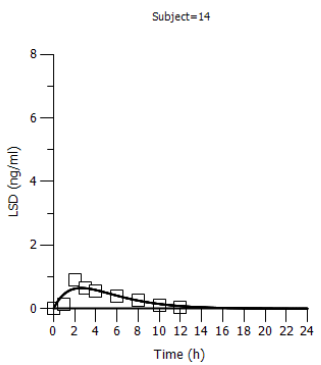
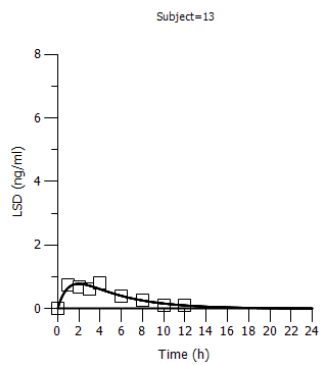
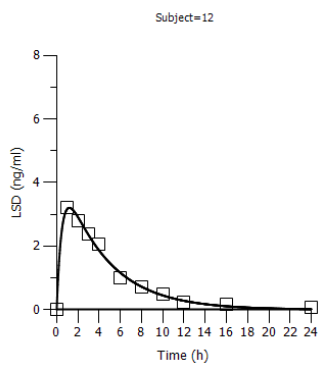
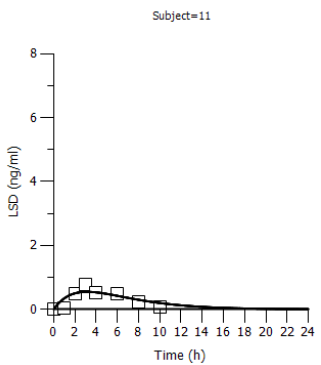
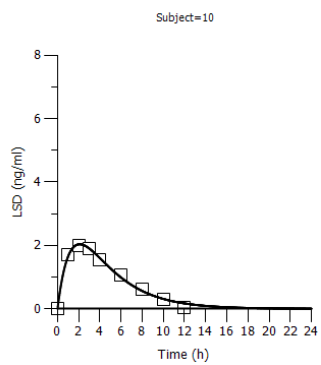
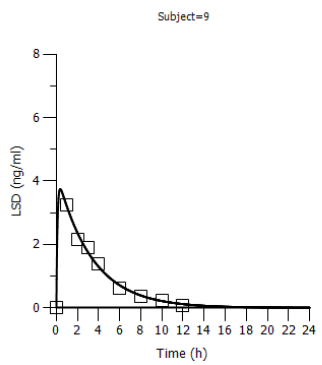
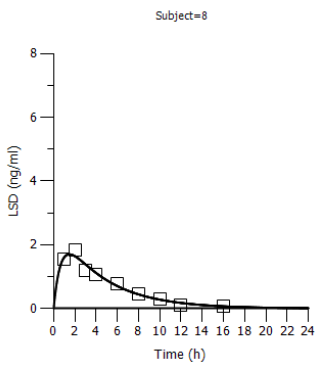
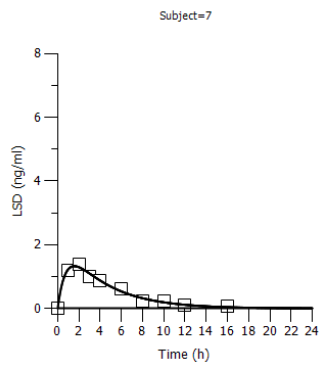
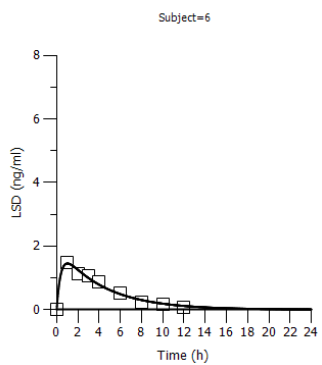
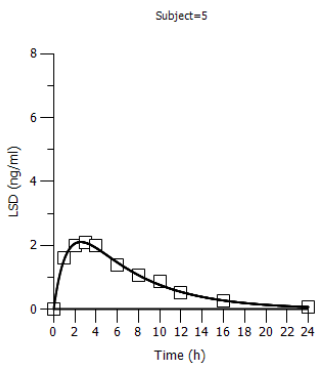
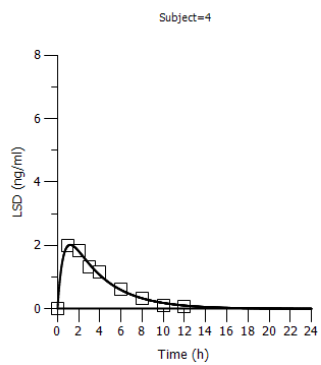
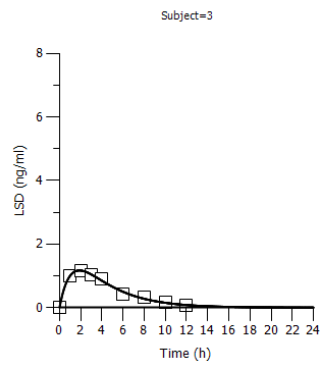
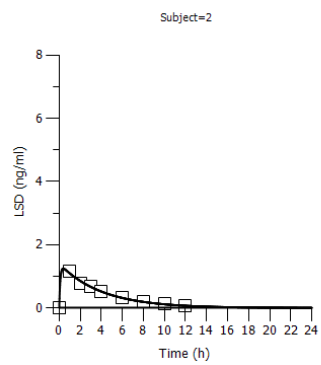
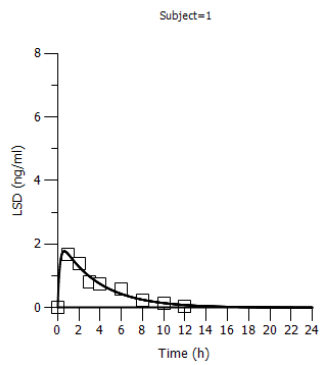
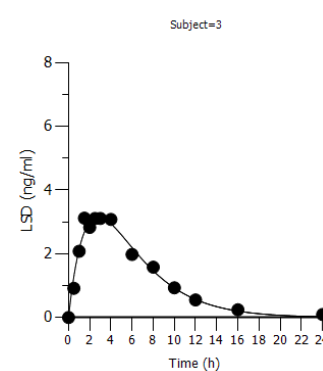
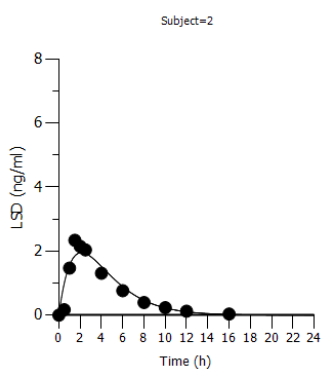
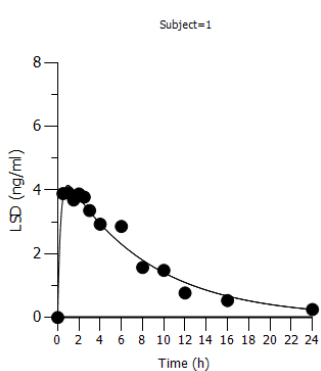
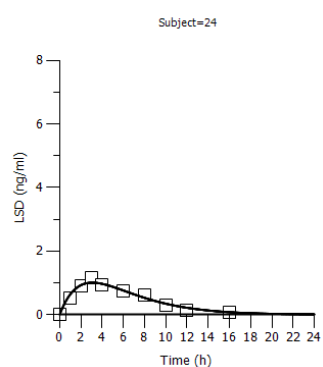
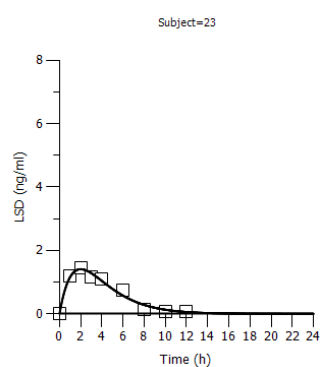
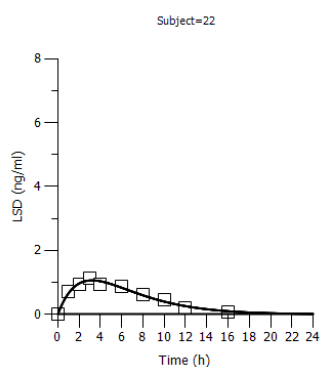
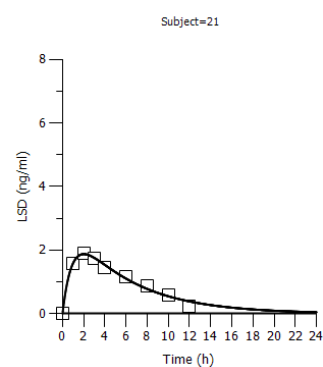
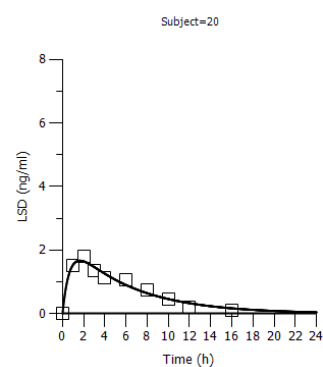
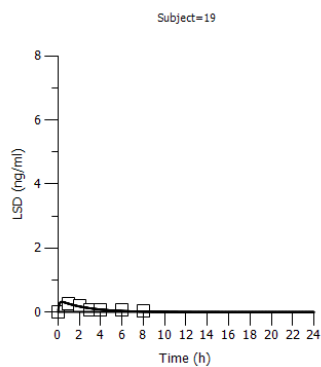
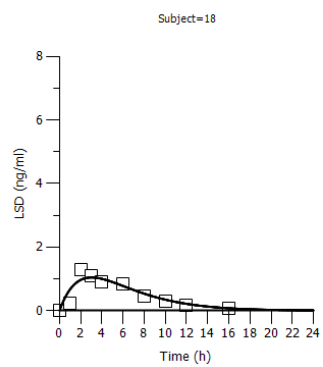
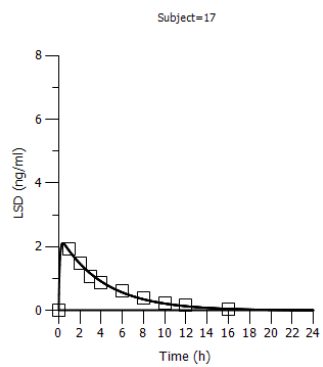
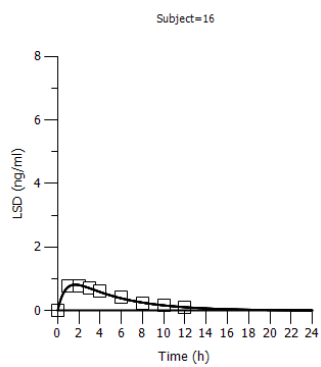


Figure S1. Schematic representation of the pharmacokinetic-pharmacodynamic link model.





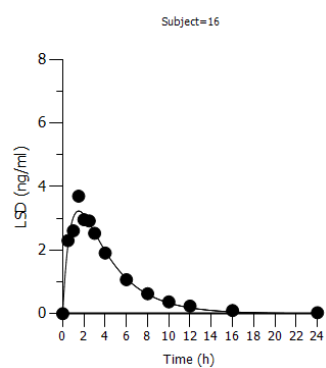
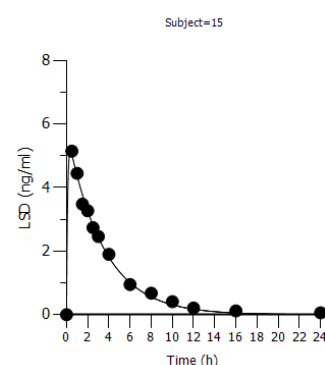
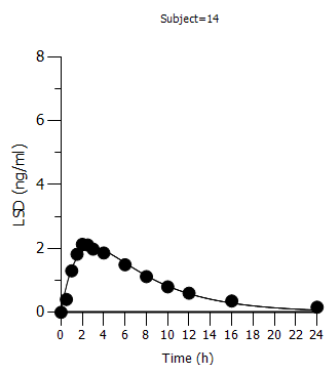
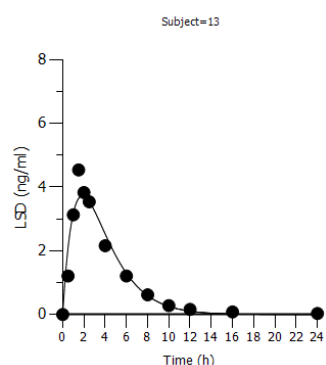
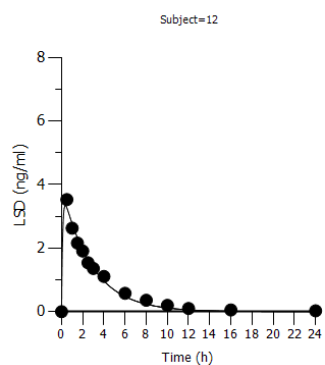
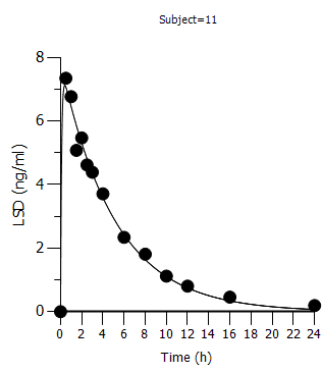
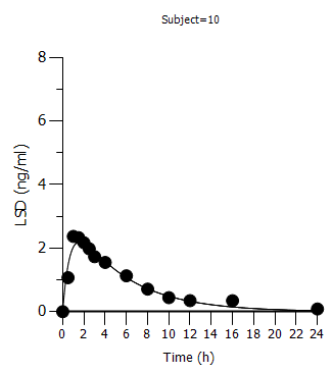
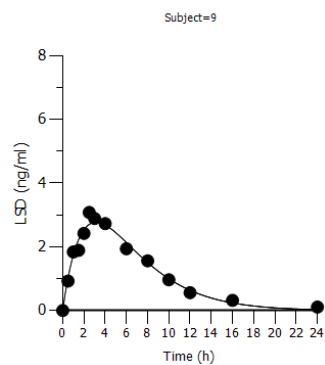
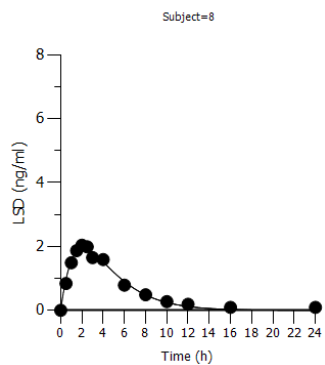
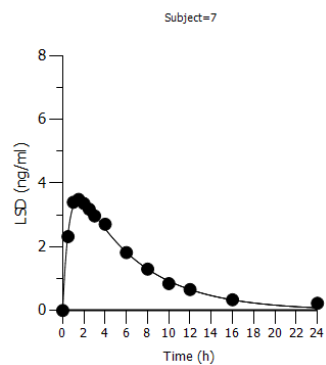
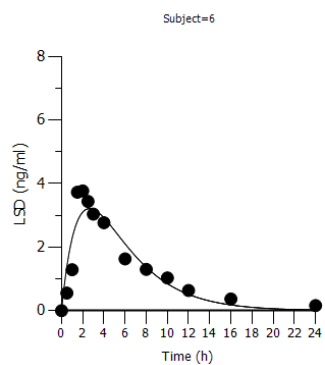
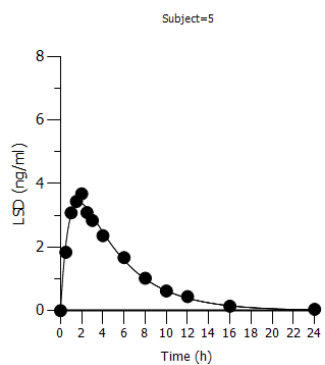
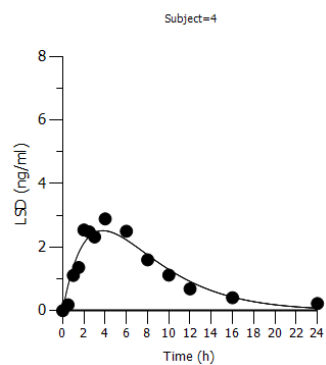


Figure S2. LSD plasma concentration-time curves. LSD was orally administered at a dose of 100 µg (panels 1-16) or 200 µg (panels 17-40) at $t = 0$. The data represent individual observed LSD plasma concentrations as measured at the different time points (□ for 100 µg and ● for 200 µg LSD) and the LSD concentrations predicted by the one-compartment pharmacokinetic model (black lines). Note the interindividual variance in plasma concentrations, especially within the 100 µg LSD dose group (panels 1-16).

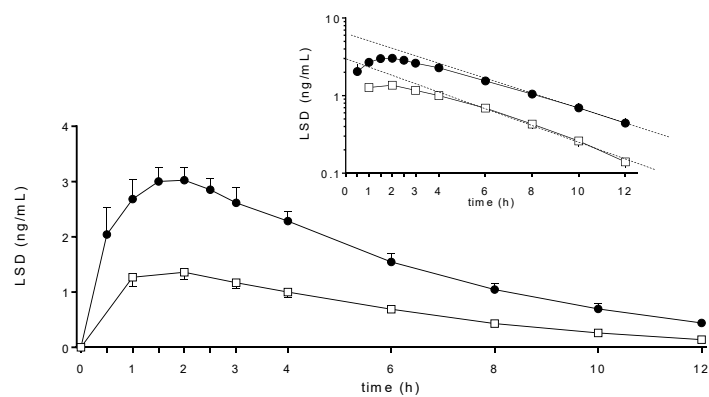
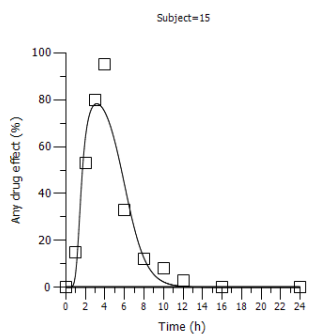
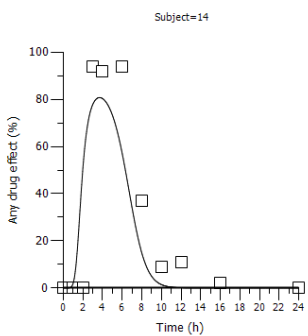
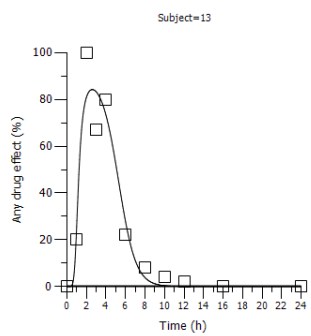
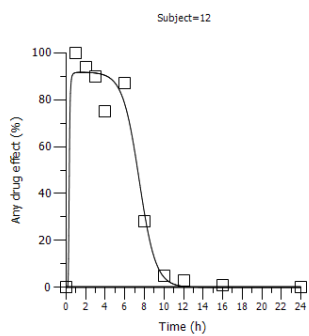
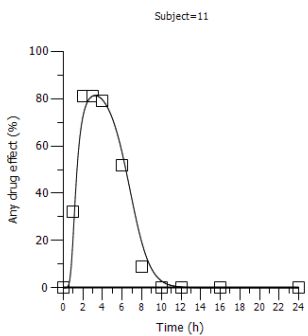
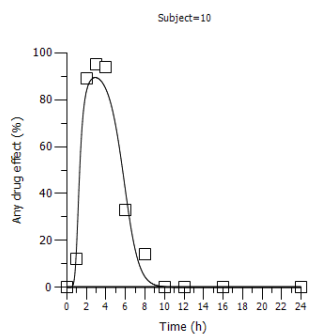
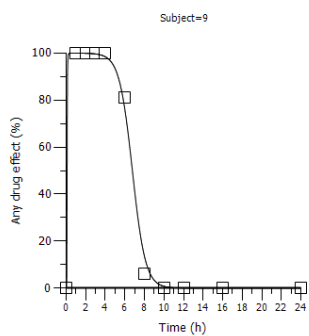
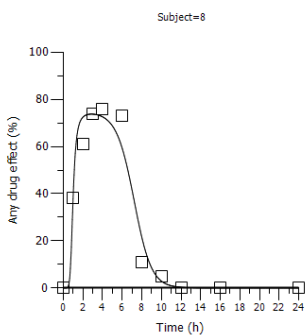
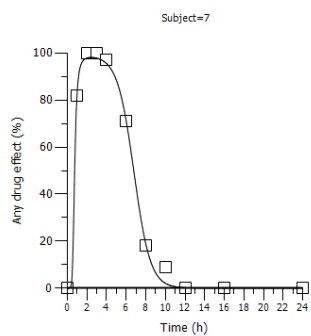
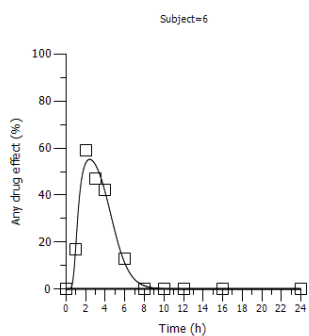
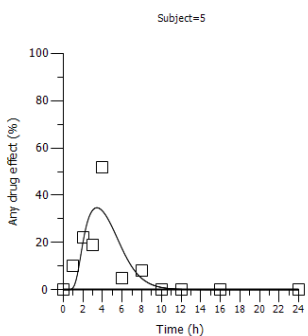
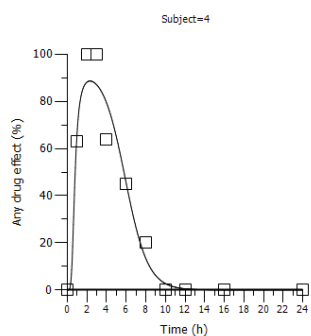
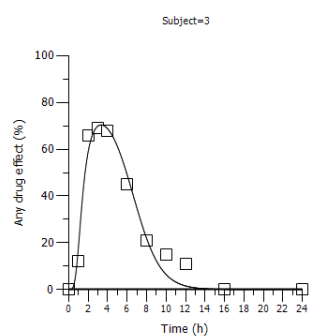
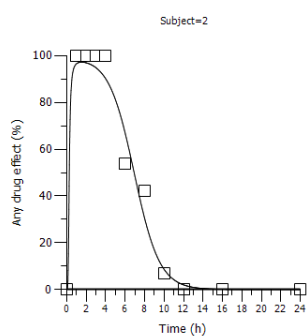
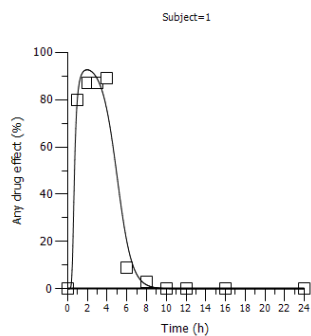
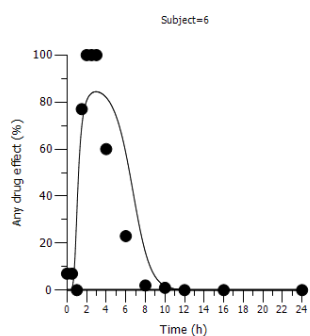
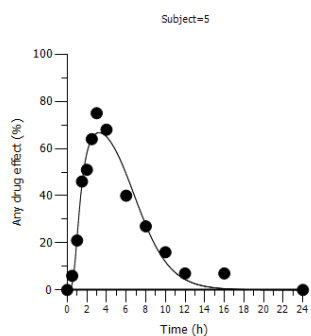
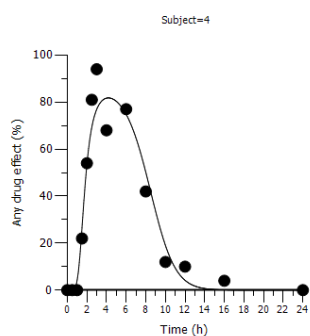
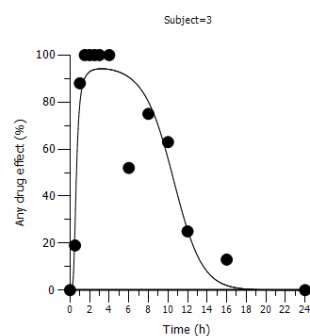
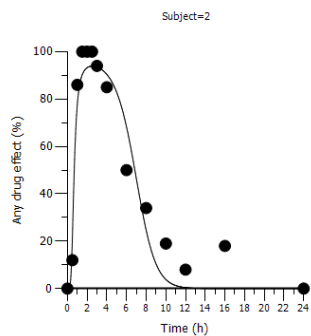
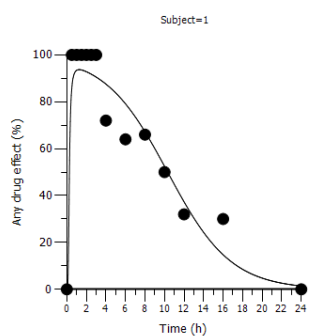
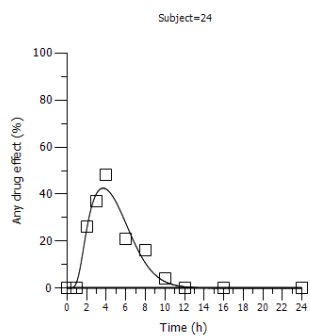
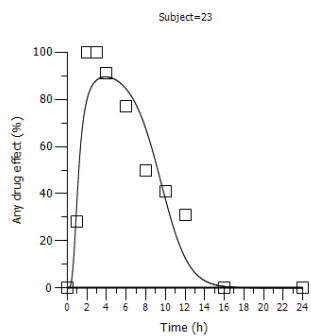
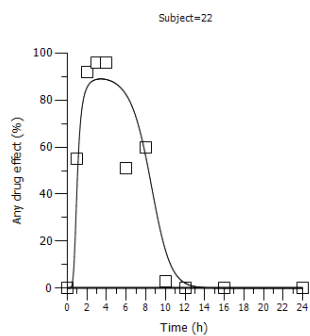
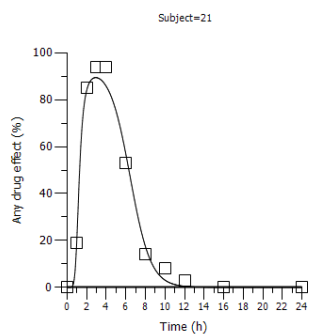
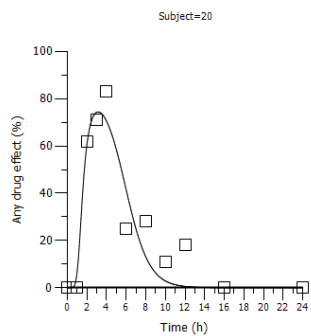
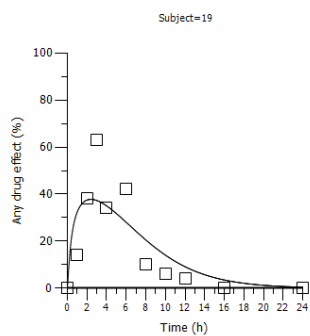
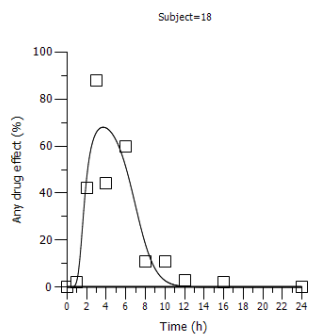
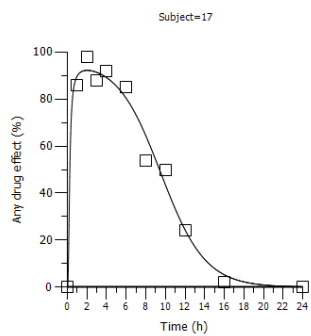
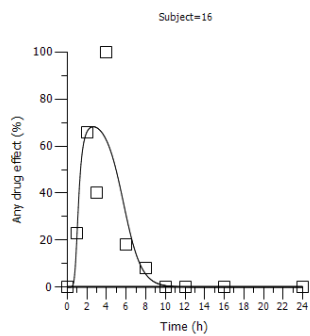


Figure S3. Plasma concentration-time curves of LSD. Filled circles (●) and empty squares (□) indicate the mean \pm SEM concentrations after 100 and 200 μ g LSD. The inset shows the semilogarithmic plot. First-order kinetics were observed up to 12 h. LSD was administered at $t = 0$.





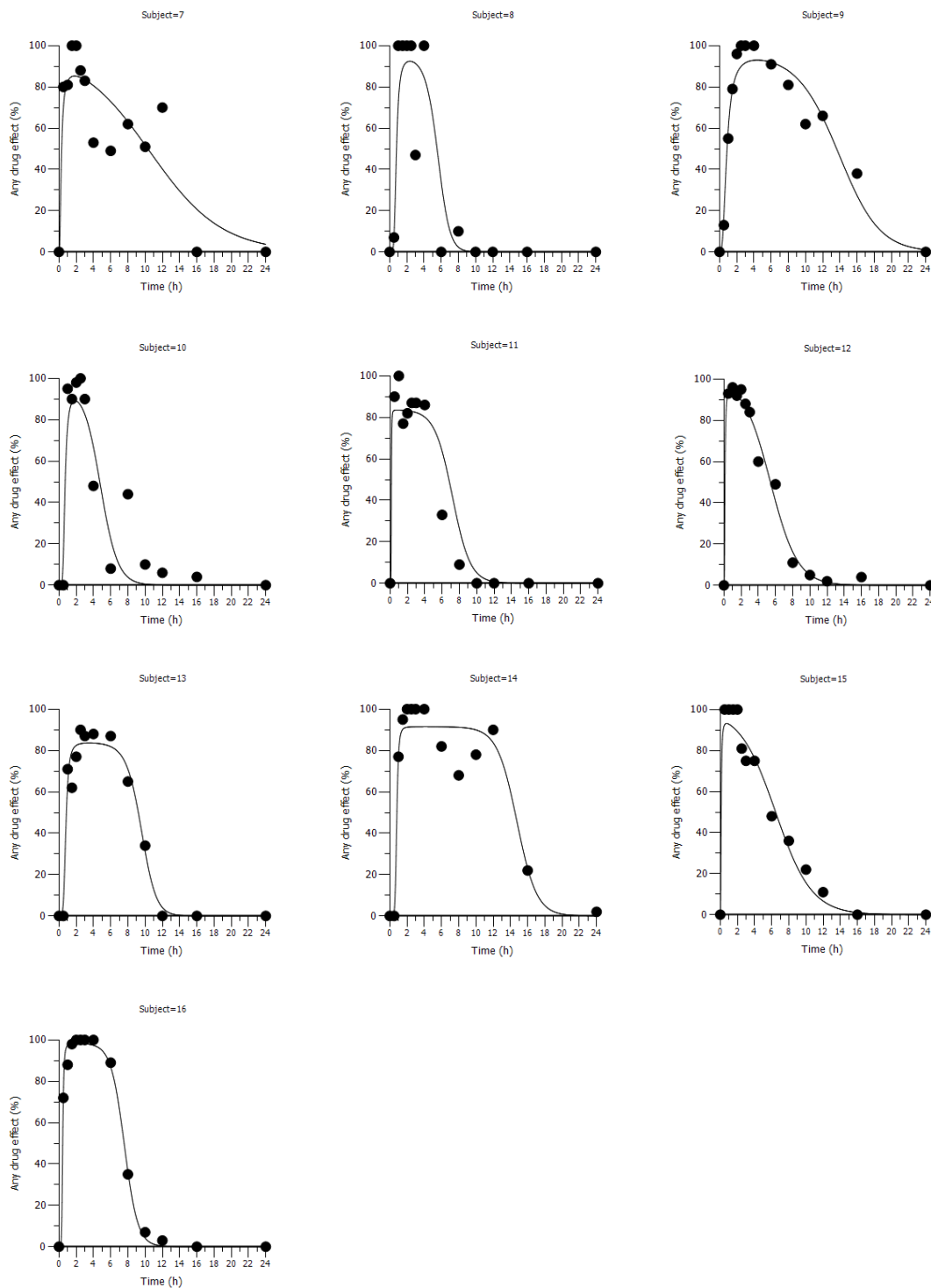
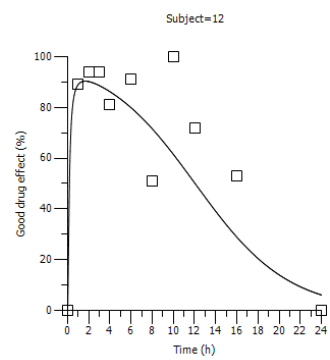
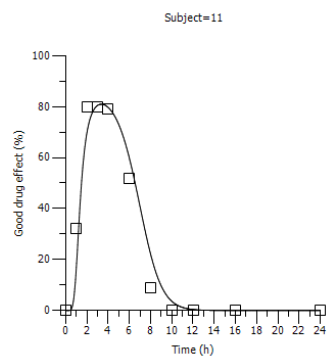
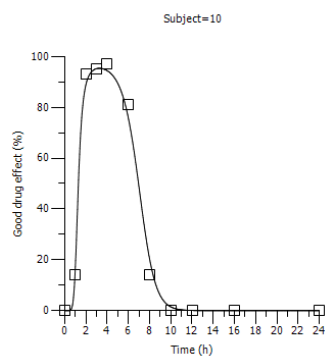
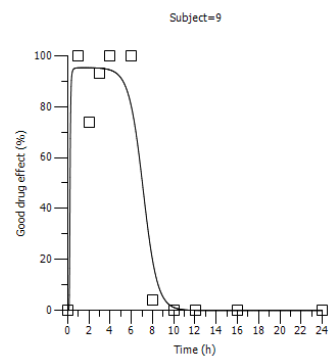
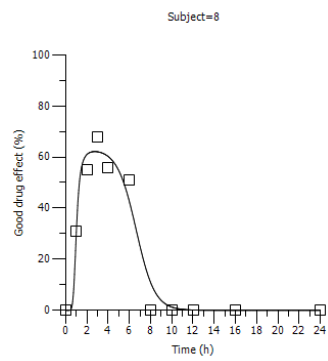
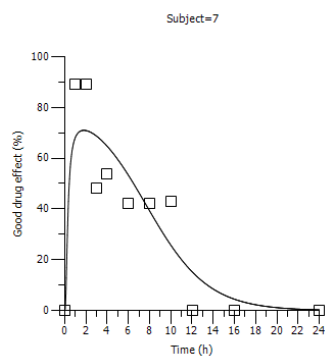
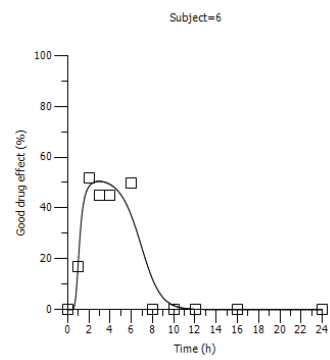
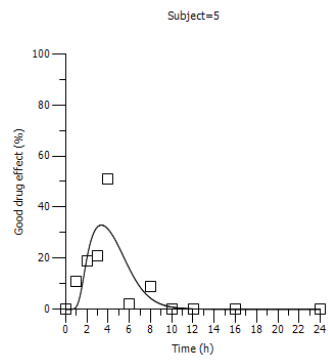
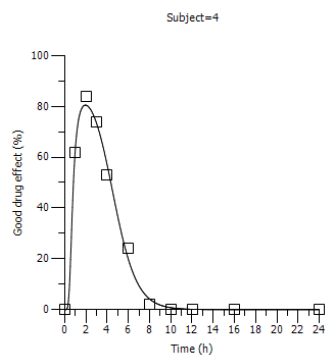
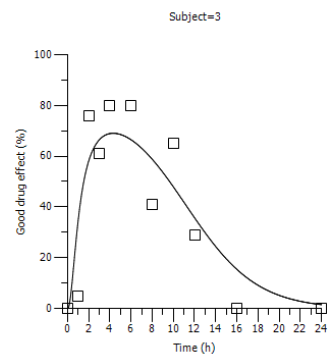
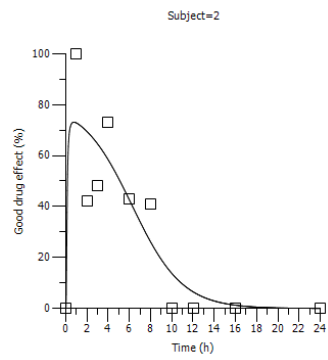
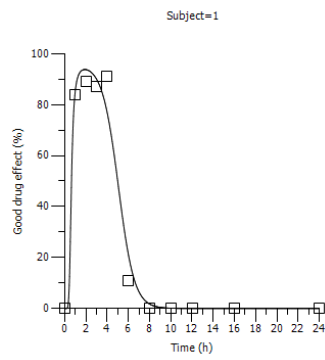
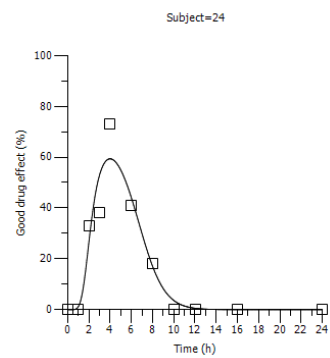
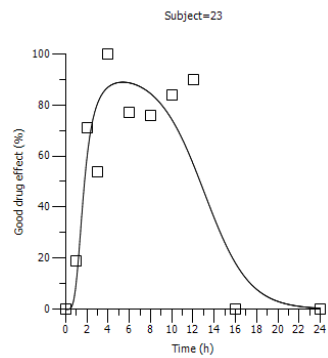
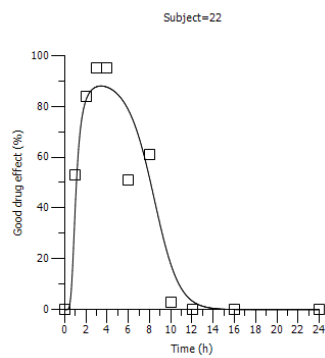
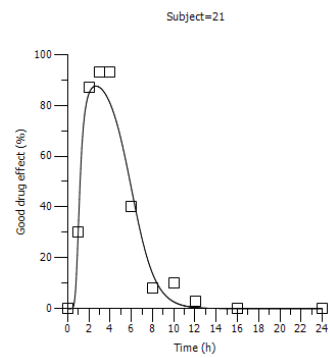
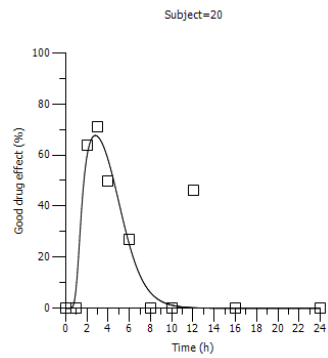
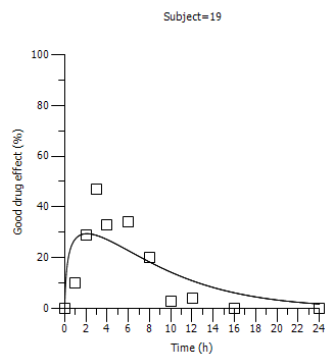
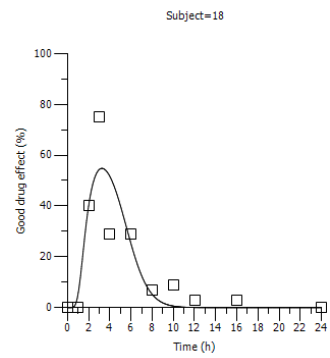
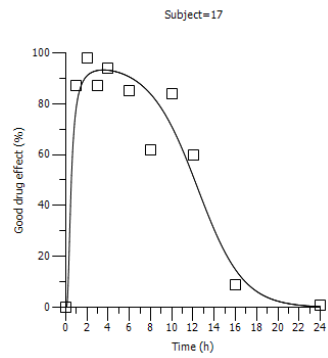
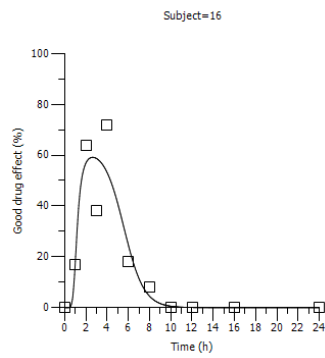
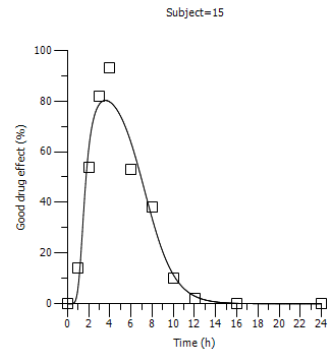
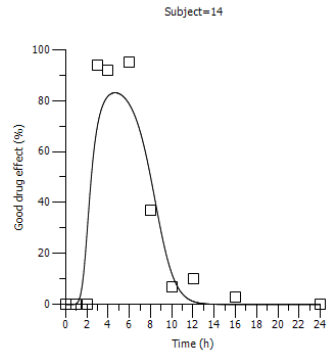
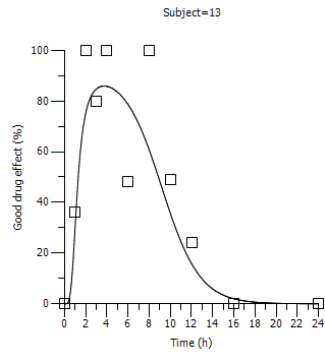
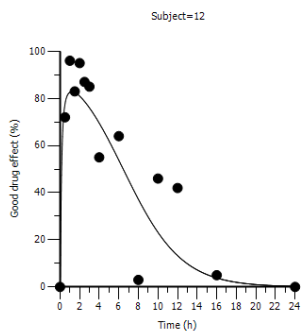
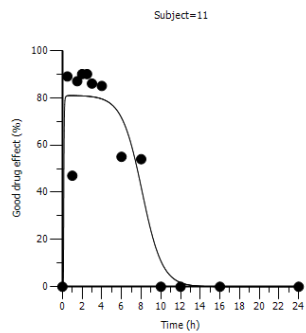
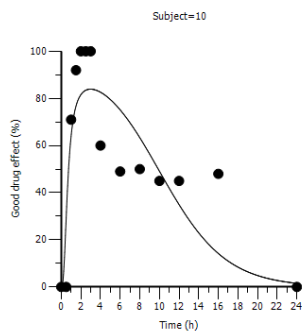
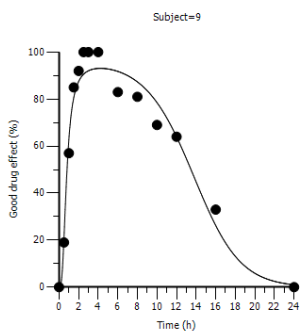
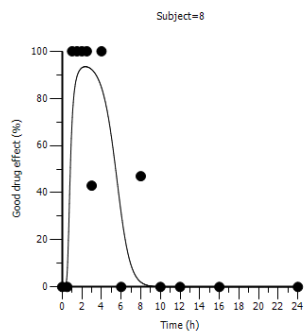
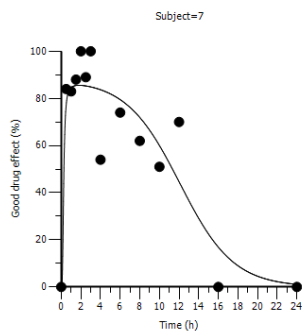
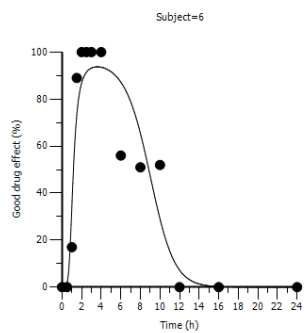
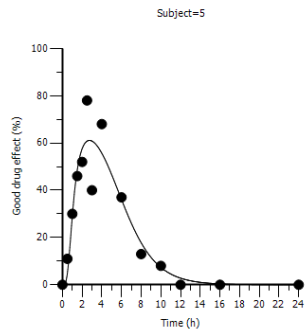
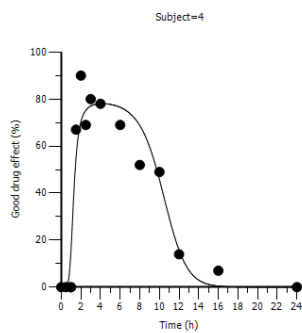
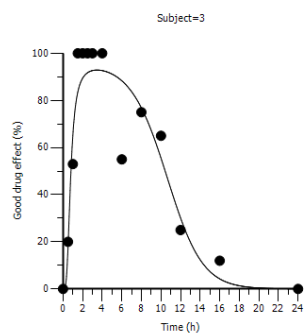
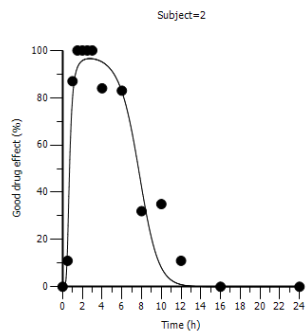
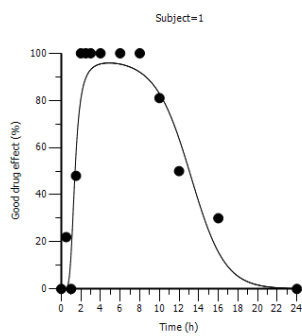


Figure S4. Subjective responses to LSD. LSD was orally administered at a dose of 100 μg (panels 1-16) or 200 μg (panels 17-40) at $t = 0$. The data represent individual observed LSD responses on the “any drug effect” Visual Analog Scale (rated 0-100%) at the different time points (\square for 100 μg LSD and \bullet for 200 μg LSD) and the pharmacokinetic-pharmacodynamic model-predicted effect (black lines).







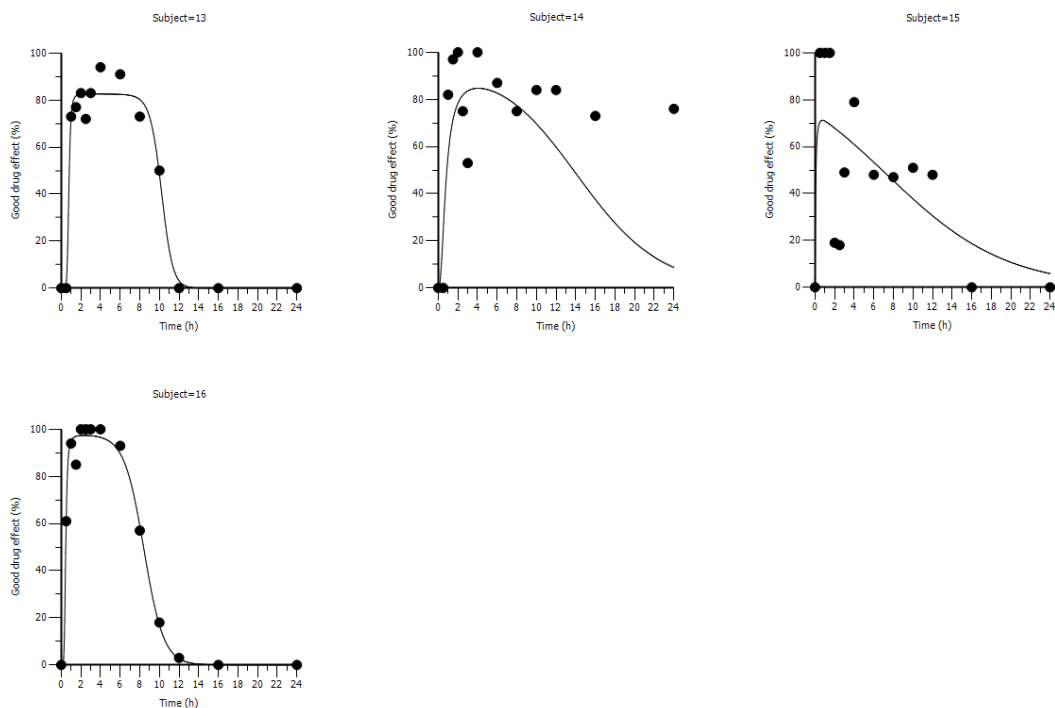
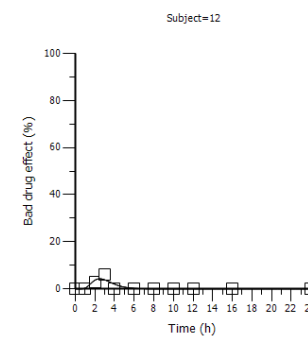
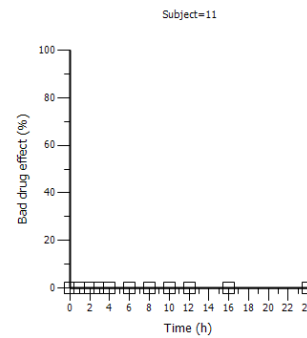
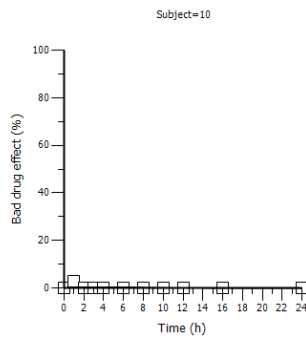
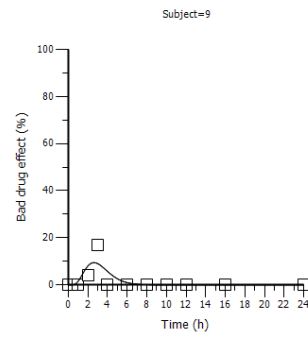
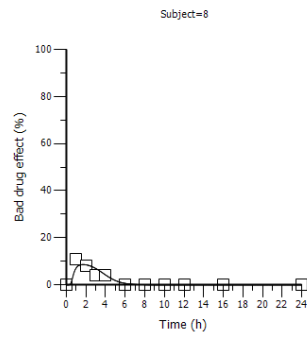
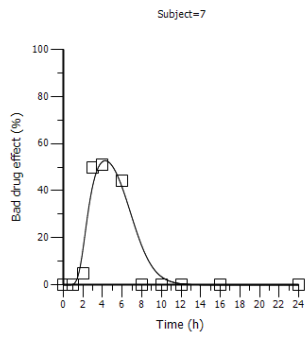
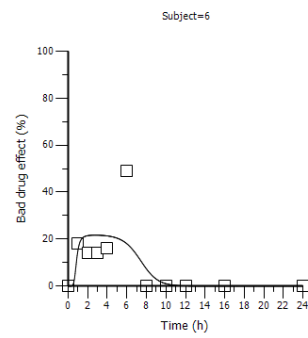
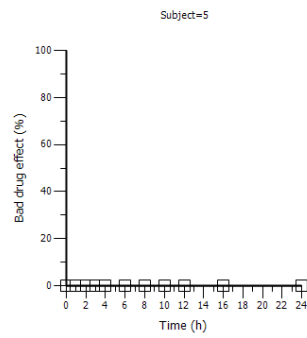
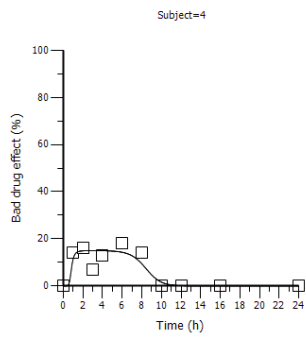
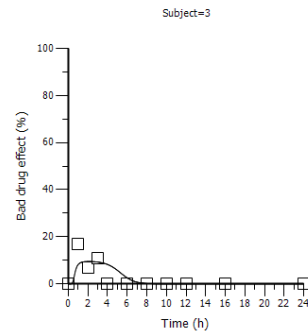
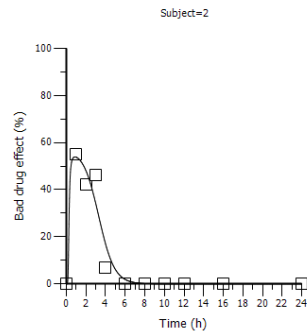
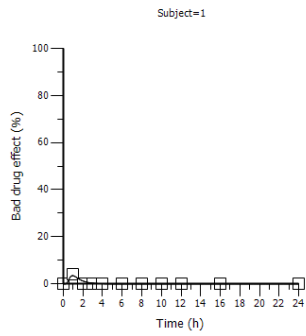
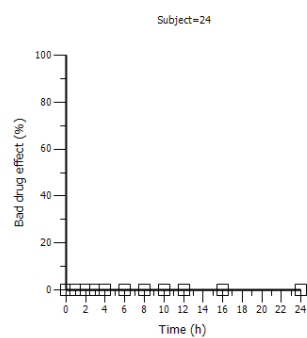
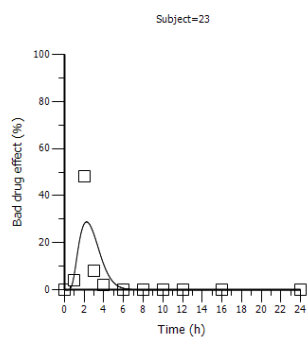
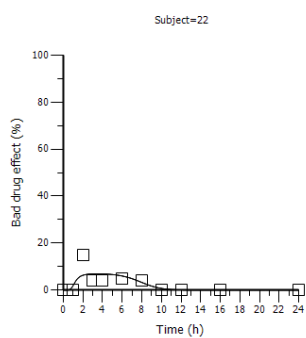
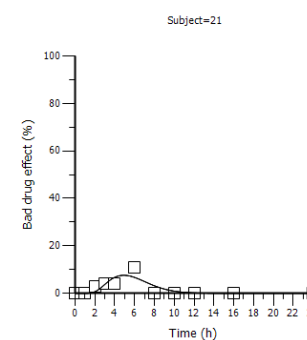
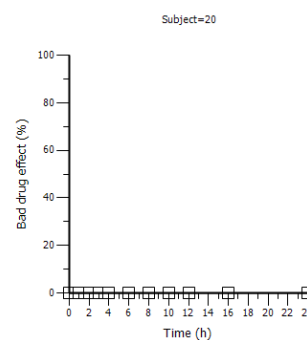
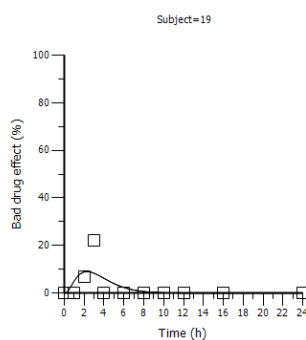
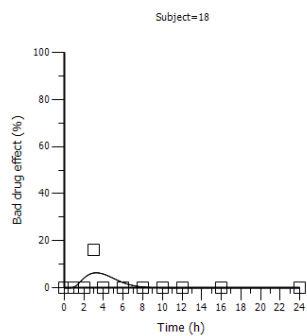
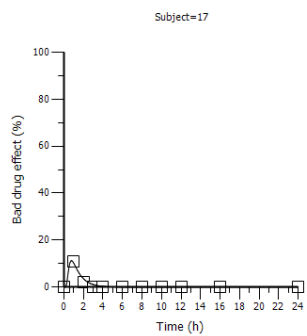
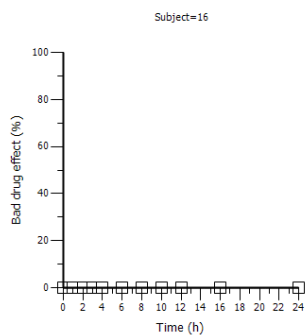
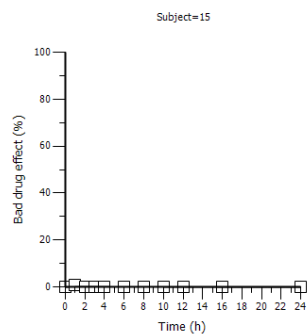
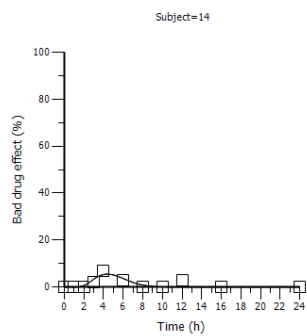
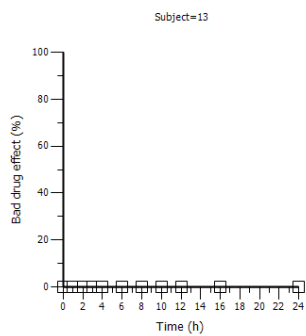
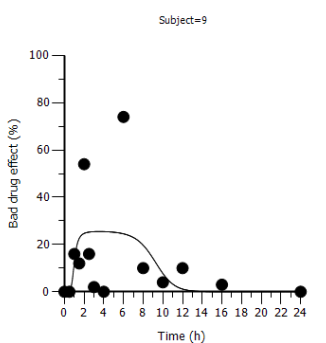
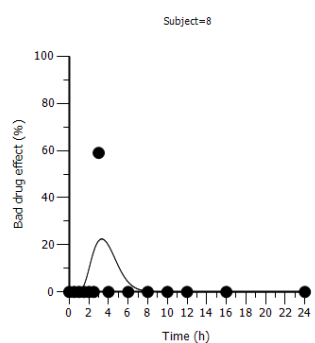
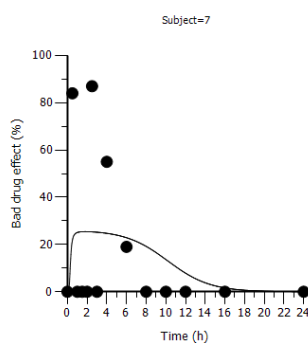
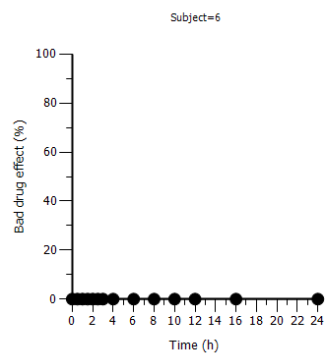
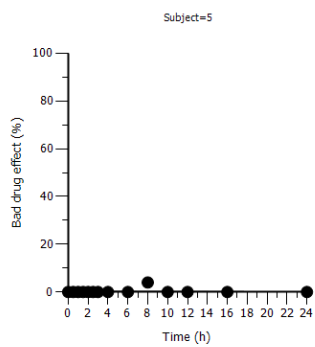
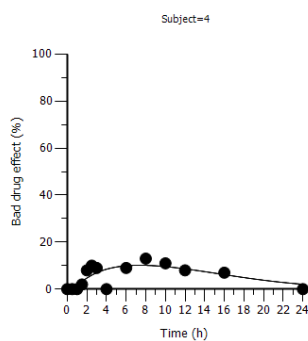
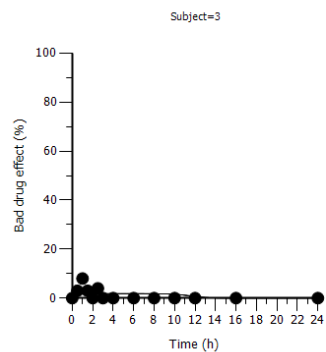
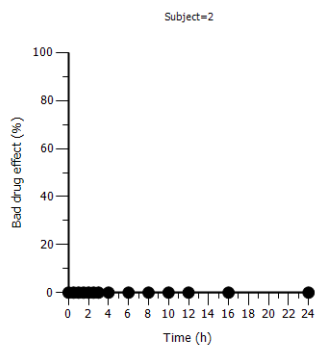
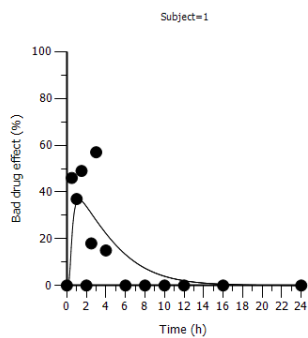


Figure S5. Subjective responses to LSD. LSD was orally administered at a dose of 100 µg (panels 1-16) or 200 µg (panels 17-40) at t = 0. The data represent individual observed LSD responses on the “good drug effect” Visual Analog Scale (rated 0-100%) at the different time points (□ for 100 µg and ● for 200 µg LSD) and the pharmacokinetic-pharmacodynamic model-predicted effect (black lines).







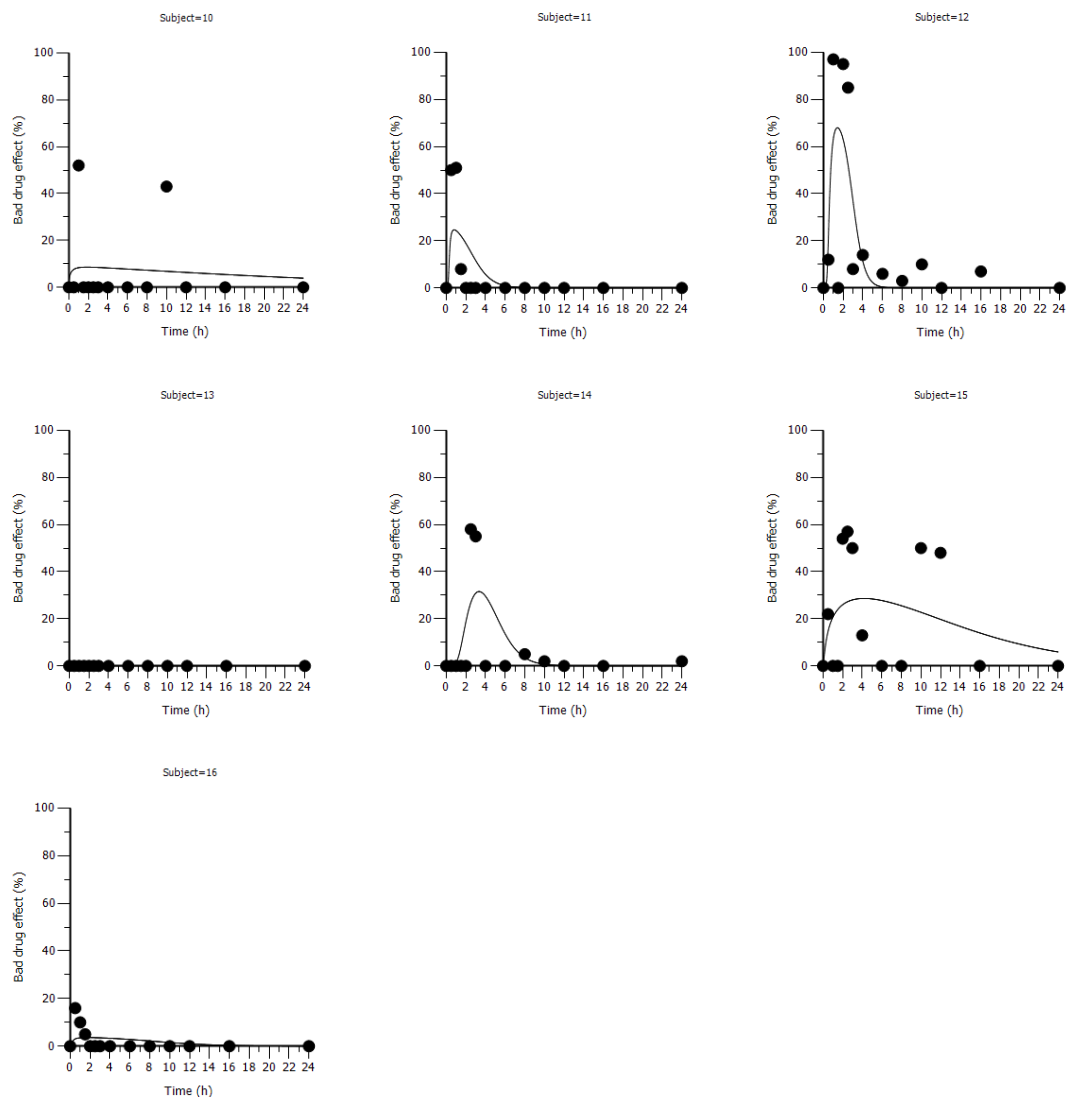


Figure S6. "Bad drug effect" of LSD. LSD was orally administered at a dose of 100 µg (panels 1-16) or 200 µg (panels 17-40) at $t = 0$. The data represent individual observed LSD responses on the "bad drug effect" Visual Analog Scale (rated 0-100%) at the different time points (□ for 100 µg and • for 200 µg LSD) and the pharmacokinetic-pharmacodynamic model-predicted effect (black lines).

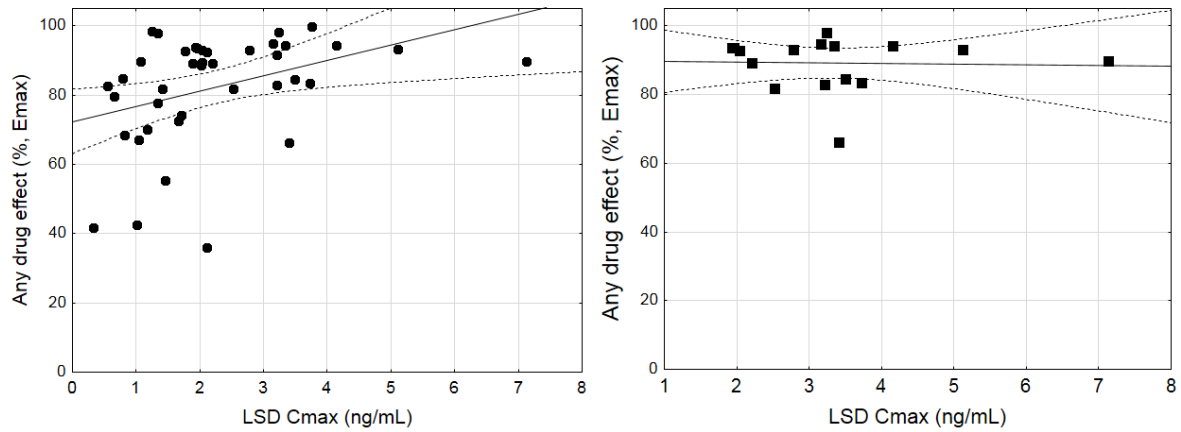


Figure S7. Association of LSD plasma concentrations (predicted Cmax levels) and peak subjective effects (predicted any drug effects) for both doses (100 and 200 µg) pooled (N=40, circles, left panel) and for the 200 µg dose alone (N=16, rectangles, right panel). Plasma concentrations of LSD are significantly correlated with its subjective effects across subjects in the pooled sample ($R_p = 0.38$, $p < 0.05$, $N = 40$, left panel). However, plasma peak concentrations are not significantly correlated with the subjective peak response within the 100 µg ($R_p = 0.38$, $p = 0.08$, $N = 24$) or the 200 µg ($R_p = -0.04$, $p > 0.8$, $N = 16$) dose groups.

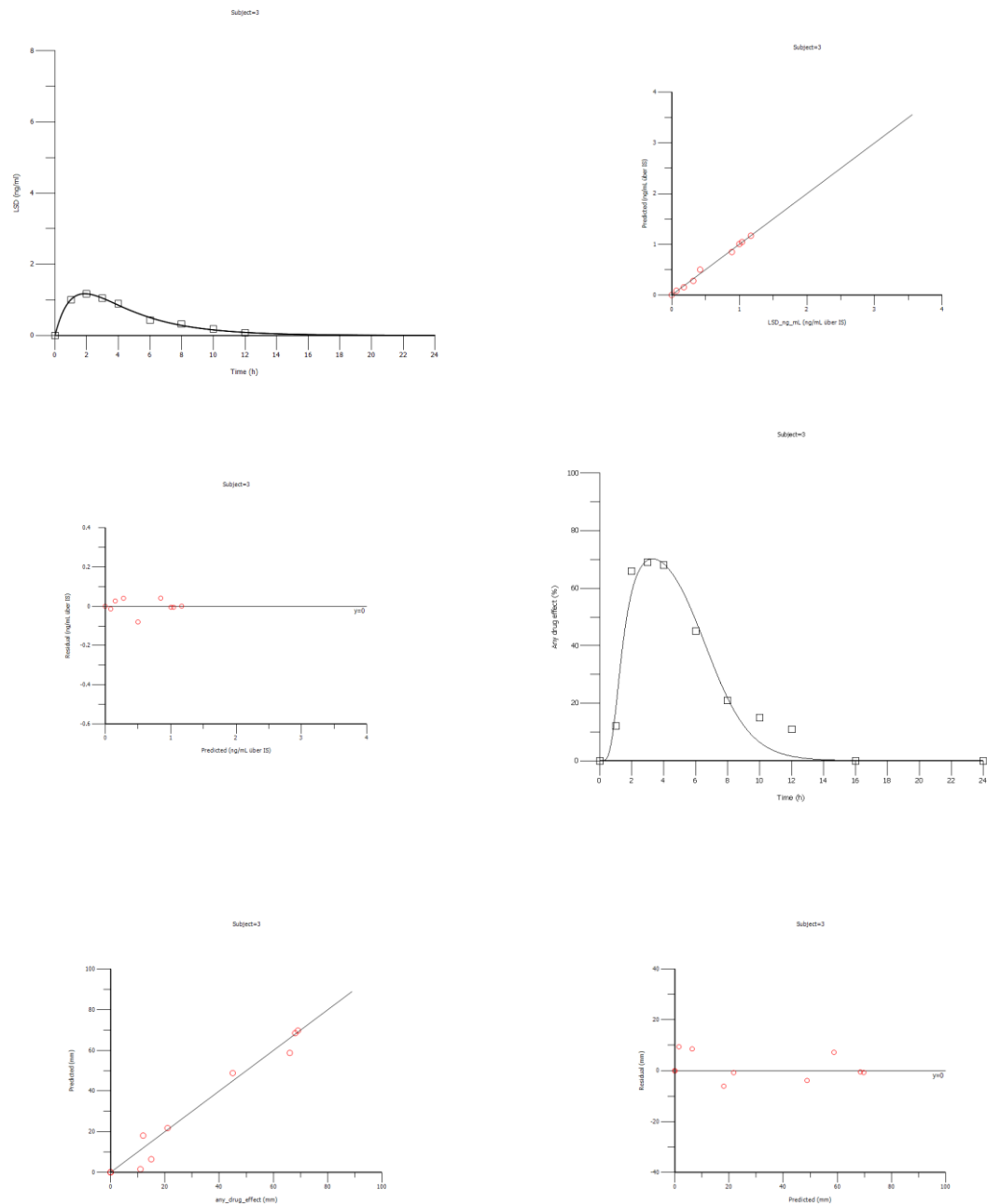


Figure S8. Diagnostic plots for a representative subject of the 100 µg LSD dose study group. upper left panel: Observed and predicted concentrations of LSD vs. time. (see Figure S2 for all plots) upper right panel: Observed vs. predicted concentrations of LSD. Middle left panel: Residual vs. predicted concentrations of LSD. Middle right panel: Observed and predicted effects of LSD vs. time. (see Figure S4 for all plots). Lower left panel: Observed vs. predicted effects of LSD. Lower right panel: Residual vs. predicted effects of LSD.

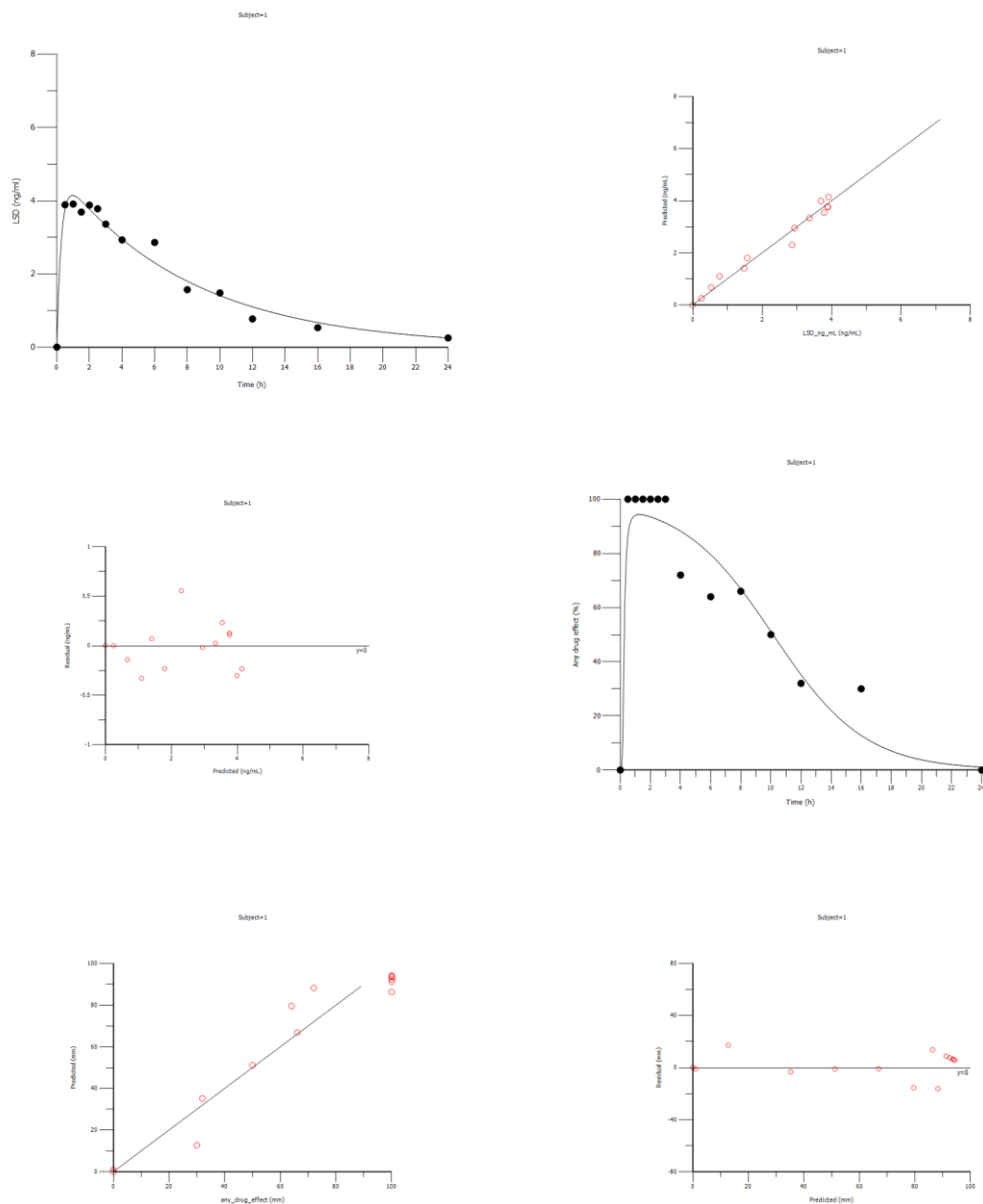


Figure S9. Diagnostic plots for a representative subject of the 200 µg LSD dose study group. upper left panel: Observed and predicted concentrations of LSD vs. time. (see Figure S2 for all plots) upper right panel: Observed vs. predicted concentrations of LSD. Middle left panel: Residual vs. predicted concentrations of LSD. Middle right panel: Observed and predicted effects of LSD vs. time. (see Figure S4 for all plots). Lower left panel: Observed vs. predicted effects of LSD. Lower right panel: Residual vs. predicted effects of LSD.

4. Discussion

4.1 Pharmacokinetics

After no research in humans since the 1970s, we have successfully conducted two double-blind, placebo-controlled, randomized, cross-over phase I studies in healthy subjects. We investigated a high dose of 200 µg of LSD in 16 subjects and a lower dose of 100 µg of LSD in 24 healthy subjects, and characterized psychological, physiological, and pharmacokinetic effects (56, 68-71).

The development of a sensitive method for the measurement of LSD and its metabolites was an analytical challenge. Due to the high potency of the substance, only very low doses are administered and thus result in very low plasma and urine concentrations. Additionally, the vulnerability of the compound to light and air demands careful handling. Therefore, we decided to evade purification procedures with solid-phase or liquid-liquid extraction. These can certainly increase the concentration and lead to better sensitivity of the LC-MS/MS method, but also form a time consuming procedure. We established a fast and reliable method for application in emergency toxicological cases where time is a crucial factor. This method was then successfully applied in five toxicology cases where consumption of LSD could be confirmed four times in serum and once in urine (65). Further, we successfully quantified concentrations of LSD, and its major (urinary) metabolite 2-oxo-3-hydroxy LSD. Following the controlled administration of 200 µg LSD in our first study, the metabolite was found to be present at around 10% of the LSD concentration in plasma, and up to 20-fold the LSD concentration in urine. Confirmation of this metabolite following the dose of 100 µg was difficult as peak plasma concentrations of LSD were around 1.5 ng/ml what corresponds to 2-oxo-3-hydroxy LSD concentrations of 0.15 ng/ml. This is already near to the limit of detection of many LC-MS/MS methods. Nonetheless, we could confirm the presence of some in-vitro identified metabolites (54) using more specific LC-MS/MS methods (67). We and another group (79) were able to detect nor-LSD, LAE, LEO, 13- or 14-hydroxy-LSD, and 2-oxo LSD in some of the plasma and urine samples after 100 and 200 µg LSD (67, 79). Nevertheless, the complete metabolic faith of LSD, including involved enzymes, is still unknown. Figure 1 gives an overview of currently identified and

possible metabolites. Future studies should address this issue and use higher doses of LSD in humans for quantification of metabolites. Further, *in vitro*/*in vivo* studies should clear up the metabolism of LSD including the involved enzyme mechanisms. Additionally, metabolites need to be commercially available to develop comprehensive analytical methods for their quantification.

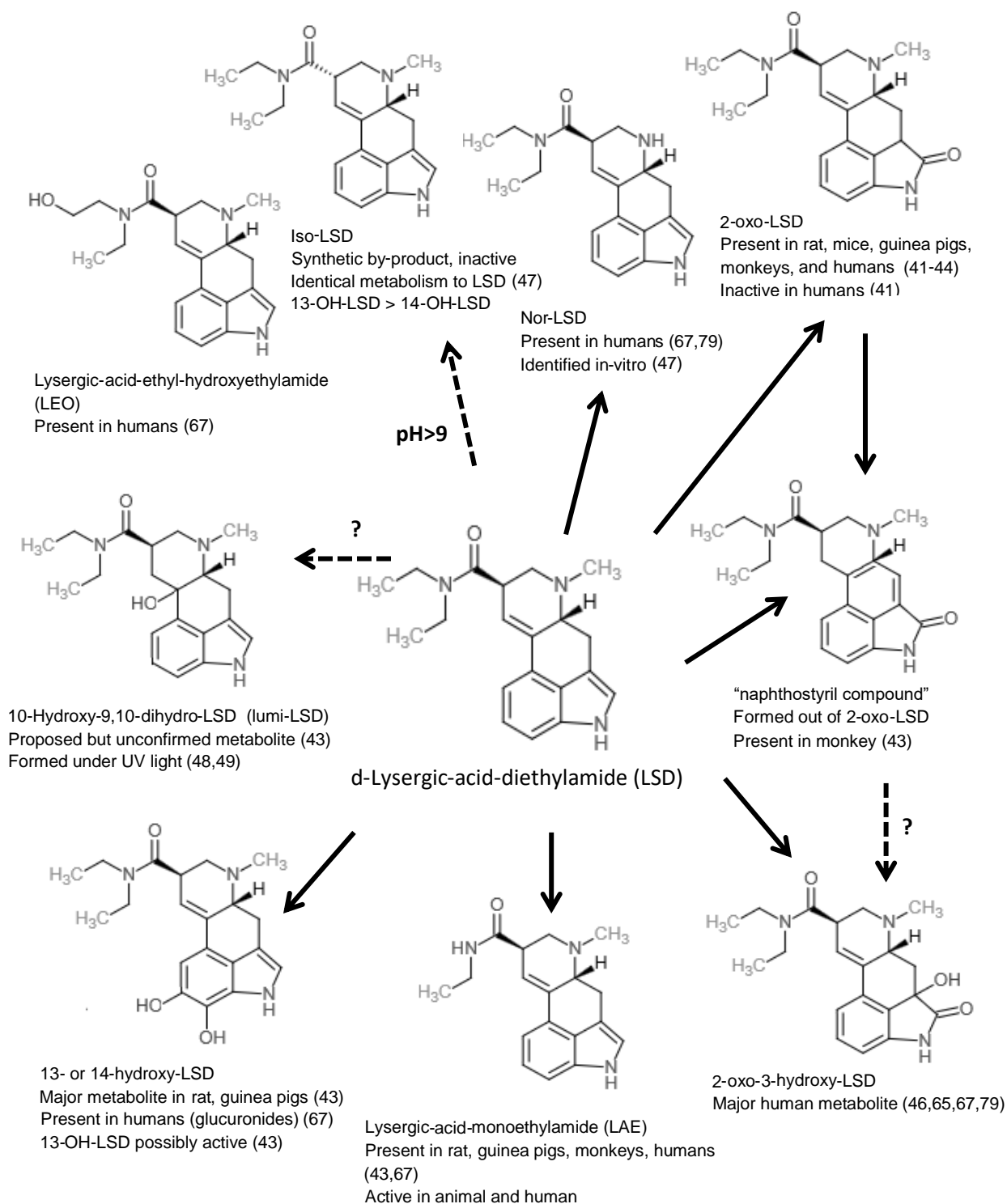


Figure 2 shows possible and already identified metabolites of LSD.

With the established LC-MS/MS methods we have assessed data on the plasma concentration-time curves of LSD. This is crucial because many experimental and therapeutic studies, some of which have started simultaneously to our studies, did not determine plasma concentrations. Thus no information on the presence of LSD in their subjects sample is available.

Maximum plasma concentrations (C_{\max}) and areas under the curve (AUC) values were approximately twice as high for the 200 μg dose compared with the 100 μg dose. *Time point of peak plasma concentrations (T_{\max}) and plasma half-lives* were similar, consistent with dose-proportional pharmacokinetics. Compartmental modeling predicted a geometric mean C_{\max} of 1.3 ng/ml, 1.4 h after the administration of 100 μg LSD. Geometric mean C_{\max} values of 3.1 ng/ml were reached 1.5 h after the administration of 200 μg LSD. The predicted mean half-life of LSD was 2.6 h after both doses and was thus comparable to the value of 2.9 hours found after intravenous administration of 2 $\mu\text{g/kg}$ LSD in the 1960s (48, 50-52) but shorter than the 3.6 hours that we have determined using non-compartmental analysis (68). Overall, we observed linear dose and elimination kinetics of LSD up to 12 hours after drug administration.

The present data on the plasma concentration-time curves of LSD are important as many studies that started investigating LSD did not perform blood sampling. There are no data available on the plasma concentrations after intravenous administration of 75 μg LSD base in saline (80), despite the publication of extensive pharmacodynamic data (75, 80-87).

4.2 Pharmacodynamics

In the present studies, LSD produced robust and high subjective drug effects in almost all of the subjects. The subjective effects lasted 8.2 ± 2.1 hours and 11.6 ± 1.7 hours (mean \pm SD) for the 100 and 200 μ g LSD doses, respectively. Subjective peak effects were reached 2.8 hours and 2.5 hours after administration of 100 and 200 μ g LSD, respectively (68, 70, 71).

Both doses of LSD induced subjective feelings of well-being, happiness, closeness to others, openness, and trust (70). LSD induced a profound altered state of consciousness on the *five dimensions of altered consciousness questionnaire* (5D-ASC) including visual hallucinations, audiovisual synesthesia, positively experienced derealisation, and depersonalization phenomena (69). These mind altering effects were dose dependent and have recently been replicated by other research groups using different doses and routes of administration (12, 69, 75) (Figure 2). Recent investigations have further shown that these alterations in consciousness are completely blocked by pretreatment with the selective 5-HT_{2A} antagonist ketanserin (12, 13). Thus, the 5-HT_{2A} receptor is the main responsible receptor, whereas others only play a minor role in LSD's mind altering effects. Further, the overall alterations of consciousness (5D-ASC total score) were significantly correlated with ratings of mystical experience on the *Mystical Effects Questionnaire* (MEQ) (69). These effects are of importance because strong mystical experiences were associated with positive long-term effects on mood and personality in healthy subjects and better therapeutic outcomes in patients with anxiety, depression, and substance use disorder in various psilocybin studies (24, 88-90). However, in our study with 200 μ g LSD, we rarely observed strong mystical experiences. This raises questions regarding expectancy effects, placebo responses, and the role of the supervisor and therapist in mystical experiences. Ratings for a "Bad drug effect" were not present in every subject and inconsistently occurred throughout the sessions and were typically mild and short lasting. Adverse effects produced by doses of 100 μ g and 200 μ g LSD mostly included complaints like difficulty in concentrating, headache, dizziness, lack of appetite, nausea, and imbalance (70). All adverse effects completely subsided within 24 - 72 hours. No severe acute adverse effects were observed in both studies and no reports of flash-back phenomena were registered.

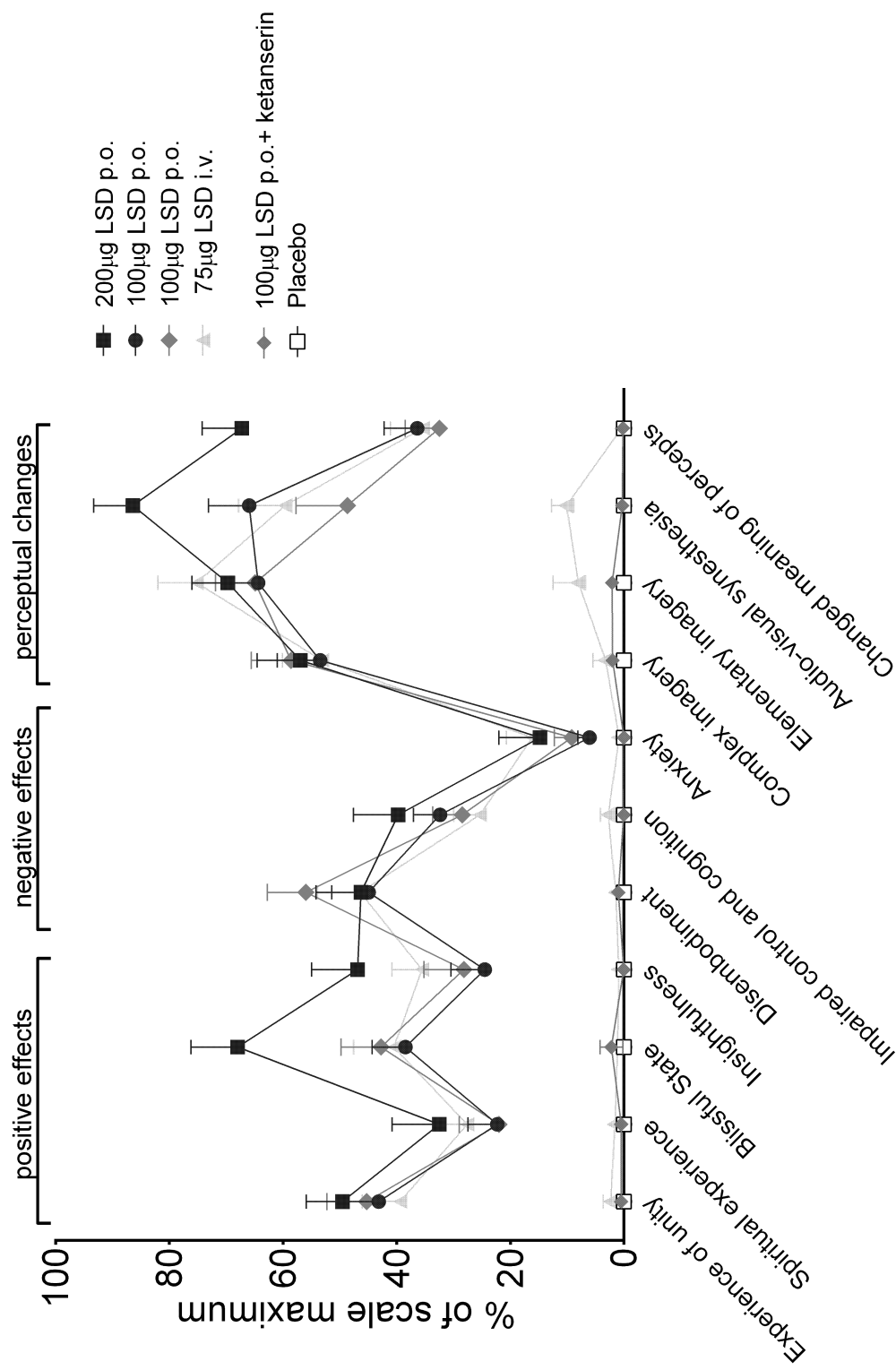


Figure 3 shows the mind altering effects of different LSD doses assessed across different research groups with the 5 dimensions of altered states of consciousness questionnaire.

200 µg LSD oral, Basel N=16, 100 µg LSD oral, Basel N=24, 100 µg LSD oral, Zürich N= 22, 75 µg LSD i.v. London N=20

4.3 Pharmacokinetics - Pharmacodynamics

The estimates of the corresponding *half maximal effective concentration* (EC_{50}) values were in the range of 0.71 - 1.2 ng/ml for positive experienced subjective effects, and between 1.5 - 2.5 ng/ml for ratings of bad drug effects (68, 71). In our studies, where relatively high and similar doses of LSD were used, the resulting plasma concentrations were above the EC_{50} of the particular response measures. Therefore responses did not vary across subjects because responses are close to the maximum. This is important to note and explains why we typically found no correlations between LSD concentrations and effects across subjects within dose groups. Probably because of the relatively high concentrations of LSD and the consistent very high subjective response ratings in most subjects. This finding needs to be considered when interpreting associations between subjective responses and other measures. However, these correlations of plasma concentrations with the subjective and cardiovascular effects of LSD are only weak during the peak response and typically the case with measures with a maximal effect limit such as subjective drug effect ratings across different questionnaires and some physiological effects like pupil size. Still we observed a close relationship between the LSD plasma concentration and subjective effects.

In both studies, we found no evidence of acute pharmacological tolerance (represented by a counterclockwise hysteresis, shown in Figure 3) within 12 hours after the 100 µg dose and within 24 hours after the 200 µg dose. In contrast, other psychoactive substances, such as *3,4-methylenedioxymethamphetamine* (MDMA), exhibit very marked acute pharmacological tolerance (represented by a clockwise hysteresis, shown in Fig 3), with a rapid decline of subjective and physiological effects of MDMA within 4 hours despite continuously high plasma levels.

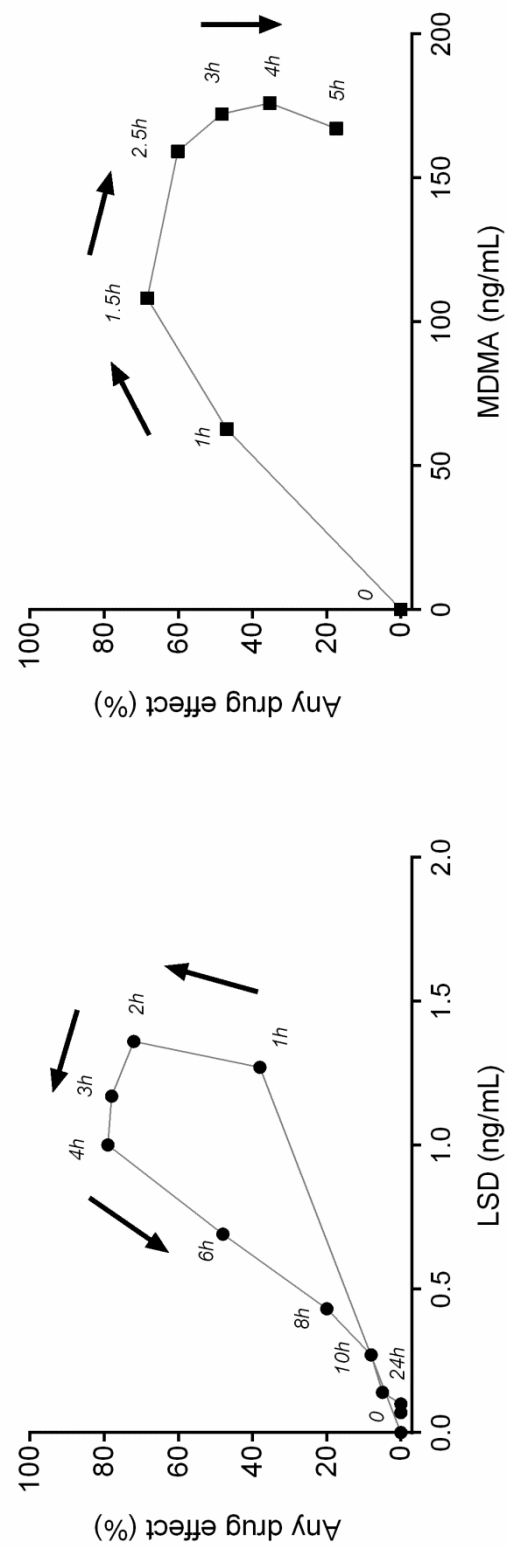


Figure 4 shows the plasma concentration – effect relationships with counterclockwise hysteresis (no acute tolerance) of LSD and clockwise hysteresis (acute tolerance) of MDMA.

4.4 Emotion Recognition and Empathy

Because of the use of LSD, mainly in psychiatric settings and the recreational use, information about the effects of LSD on social cognition are important. Social cognition includes aspects of emotion recognition and empathy which describe the ability to infer another's thoughts, feelings, and intentions. Those are relevant for a better understanding of the human brain structures and functioning, as well as simple social interactions during clinical studies and mainly in psychotherapeutic settings. Results from earlier studies were primarily observational and thus very subjective.

To allow for a better characterization of the social-cognitive effects of LSD, we used validated psychometric instruments that have been used with other drugs, such as MDMA, methylphenidate, and psilocybin (72-74, 76, 77).

Interestingly, 100 and 200 µg LSD positively altered the processing of emotional information by decreasing the recognition of fearful faces and tended to impair the recognition of sad faces (70). Further, 200 µg LSD significantly enhanced emotional empathy whereas the effects of 100 µg LSD did nearly not reach significance.

Overall, these effects are similar to those observed following MDMA administration which similarly impaired the correct recognition of negative emotions and induced strong feelings of well-being, and empathy. Similar to LSD, psilocybin decreased the recognition of negative facial expressions and increased emotional empathy (76, 77). These findings indicate that LSD affects emotion processing similarly to MDMA and psilocybin. In line with the findings of impaired recognition of fear following LSD administration, we found that 100 µg of LSD reduced left amygdala reactivity to the presentation of fearful faces relative to placebo (78). Similarly, psilocybin and MDMA decreased amygdala reactivity to negative facial stimuli (91, 92).

The emotional effects during the later phase of the acute LSD response (6-10 h) and the reduced perception of negative emotions/amygdala reactivity are likely beneficial in psychotherapeutic settings. Future research should therefore address the relative contributions of the empathic and emotional effects of LSD to its potential therapeutic effects.

5. Summary and Outlook

The findings of the two clinical studies about the effects of LSD in healthy participants have translational relevance for further medical investigations. First, we have shown that LSD can be safely administered to healthy subjects when closely monitored and supervised by experienced investigators. Second, the PK-PD relationship shows that the subjective effects are directly related to the plasma concentrations and that LSD does not produce acute tolerance. Third, the increase in emotional empathy and the bias towards the recognition of positive emotions in line with a decreased amygdala reactivity, might reflect a potentially therapeutic effect by reducing perception of negative emotions and facilitating the therapeutic alliance in LSD-assisted psychotherapy (e.g. in anxiety disorders). Currently LSD-assisted psychotherapy is offered by two psychiatrists to selected patients in Switzerland in the context of compassionate use which is legally-authorized, but demands case-by-case authorization by the Swiss Federal Office for Public Health (BAG).

Due to the study results and the pioneer work in the field of compassionate use, we were able to get the approval for a new clinical phase II study, investigating LSD in 40 patients with anxiety (with or without life threatening diseases). Because the higher dose of 200 µg LSD produced stronger subjective and emotional effects while having comparable cardiovascular stimulation as the lower 100 µg dose, it was selected to be used in this phase II trial. The study has just started and uses a double-blind, placebo-controlled, within-subject, cross-over design with two LSD and two placebo sessions. Our hypothesis is that LSD will significantly reduce anxiety in these patients. The study will last until 2021.

References

1. Hollister LE. Human pharmacology of lysergic acid diethylamide. *Psychopharmacology - A review of progress 1957 - 1967*. 1967;Proceedings of the 6th annual meeting of the ACNP. San Juan, Puerto Rico. Dec. 12-15, 1967:1253.
2. Hoffer A. D-Lysergic Acid Diethylamide (LSD): A Review of its Present Status. *Clin Pharmacol Ther*. 1965;6:183.
3. Victor Rossi G. LSD - A pharmacologic profile. *Amer.J.Pharm*. 1971;143:38.
4. Greiner T, Burch N, Edelberg R. Threshold doses of LSD in human subjects. *Fed. Proc*. 1957;16:303.
5. Abramson HA, Jarvik ME, Kaufman MR, Kornetsky C, Levine A, Wagner M. Lysergic Acid Diethylamide (LSD-25): I. Physiological and Perceptual Responses. *The Journal of Psychology*. 1955;39(1):3.
6. Watts VJ, Lawler CP, Fox DR, Neve KA, Nichols DE, Mailman RB. LSD and structural analogs: pharmacological evaluation at D1 dopamine receptors. *Psychopharmacology (Berl)*. 1995;118(4):401.
7. Giacomelli S, Palmery M, Romanelli L, Cheng CY, Silvestrini B. Lysergic acid diethylamide (LSD) is a partial agonist of D2 dopaminergic receptors and it potentiates dopamine-mediated prolactin secretion in lactotrophs in vitro. *Life Sci*. 1998;63(3):215.
8. Halberstadt AL, Geyer MA. Multiple receptors contribute to the behavioral effects of indoleamine hallucinogens. *Neuropharmacology*. 2011;61(3):364.
9. Nichols DE. Hallucinogens. *Pharmacol Ther*. 2004;101(2):131.
10. Egan CT, Herrick-Davis K, Miller K, Glennon RA, Teitler M. Agonist activity of LSD and lisuride at cloned 5HT_{2A} and 5HT_{2C} receptors. *Psychopharmacology (Berl)*. 1998;136(4):409.
11. Gresch PJ, Barrett RJ, Sanders-Bush E, Smith RL. 5-Hydroxytryptamine (serotonin)_{2A} receptors in rat anterior cingulate cortex mediate the discriminative stimulus properties of d-lysergic acid diethylamide. *J Pharmacol Exp Ther*. 2007;320(2):662.
12. Preller KH, Herdener M, Pokorny T, Planzer A, Kraehenmann R, Stampfli P, et al. The Fabric of Meaning and Subjective Effects in LSD-Induced States Depend on Serotonin 2A Receptor Activation. *Curr Biol*. 2017;27(3):451.

13. Kraehenmann R, Pokorny D, Vollenweider L, Preller KH, Pokorny T, Seifritz E, et al. Dreamlike effects of LSD on waking imagery in humans depend on serotonin 2A receptor activation. *Psychopharmacology (Berl)*. 2017;10.1007/s00213-017-4610-0.
14. Passie T, Halpern JH, Stichtenoth DO, Emrich HM, Hintzen A. The pharmacology of lysergic acid diethylamide: a review. *CNS Neurosci Ther*. 2008;14(4):295.
15. Abramson HA, Jarvik ME, Gorin MH, Hirsch MW. Lysergic Acid Diethylamide (LSD-25): XVII. Tolerance Development and its Relationship to a Theory of Psychosis. *The Journal of Psychology*. 1956;41(1):81.
16. Isbell H, Fraser H, Wikler A, Belleville R. Tolerance to diethylamide of lysergic acid (LSD-25). *Fed. Proc*. 1955;14:355.
17. Isbell H. Comparison of the reactions induced by psilocybin and LSD-25 in man. *Psychopharmacologia*. 1959;1(1):29.
18. Abramson HA, Rolo A, Sklarofsky B, Stache J. Production of Cross-Tolerance to Psychosis-Producing Doses of Lysergic Acid Diethylamide and Psilocybin. *The Journal of Psychology*. 1960;49(1):151.
19. McGlothlin WH, Arnold DO, Freedman DX. Organicity measures following repeated lsd ingestion. *Archives of General Psychiatry*. 1969;21(6):704.
20. Anastasopoulos G, Photiades H. Effects of LSD-25 on Relatives of Schizophrenic Patients. *The British Journal of Psychiatry*. 1962;108(452):95.
21. McGlothlin W, Cohen S, McGlothlin MS. Long lasting effects of LSD on normals. *Arch Gen Psychiatry*. 1967;17(5):521.
22. Hofmann A. How LSD originated. *J Psychedelic Drugs*. 1979;11(1-2):53.
23. Nichols DE. Psychedelics. *Pharmacol Rev*. 2016;68(2):264.
24. Nichols DE, Johnson MW, Nichols CD. Psychedelics as Medicines: An Emerging New Paradigm. *Clin Pharmacol Ther*. 2017;101(2):209.
25. Pahnke WN, Kurland AA, Unger S, Savage C, Grof S. The experimental use of psychedelic (LSD) psychotherapy. *Jama*. 1970;212(11):1856.
26. Grof S, Goodman LE, Richards WA, Kurland AA. LSD-assisted psychotherapy in patients with terminal cancer. *Int Pharmacopsychiatry*. 1973;8(3):129.
27. Geyer MA, Vollenweider FX. Serotonin research: contributions to understanding psychoses. *Trends Pharmacol Sci*. 2008;29(9):445.

28. Halberstadt AL, Geyer MA. Serotonergic hallucinogens as translational models relevant to schizophrenia. *Int J Neuropsychopharmacol*. 2013;16(10):2165.
29. Anderson EW, Rawsley K. Clinical studies of lysergic acid diethylamide. *Monatsschr Psychiatr Neurol*. 1954;128(1-2):38.
30. Stoll WA. Lysergsäureäthylamid, ein Phantastikum aus der Mutterkorngruppe. *Schweiz Arch Neurol Psychiatr*. 1947;60:279.
31. Chwelos N, Blewett D, Smith C, Hoffer A. Use of d-lysergic acid diethylamide in the treatment of alcoholism. *QJ Stud Alcohol*. 1959;20:577.
32. Pahnke WN, Kurland AA, Goodman LE, Richards WA. LSD-assisted psychotherapy with terminal cancer patients. *Curr Psychiatr Ther*. 1969;9:144.
33. Kast E. LSD and the dying patient. *The Chicago Medical School quarterly*. 1965;26(2):80.
34. Raffaelli E, Martins OJ, Dágua Filho AdSP. Lisuride in cluster headache. *Headache: The Journal of Head and Face Pain*. 1983;23(3):117.
35. Cheek FE. Exploratory study of drugs and social interaction. *Archives of General Psychiatry*. 1963;9(6):566.
36. Cheek FE, Holstein CM. Lysergic acid diethylamide tartrate (LSD-25) dosage levels, group differences and social interaction. *J Nerv Ment Dis*. 1971;153(2):133.
37. Levine A, Abramson HA, Kaufman MR, Kornetsky C, Markham S. Lysergic acid diethylamide (LSD-25): XIV. Effect on personality as observed in psychological tests. *Journal of Psychology.. - Provincetown, Mass., etc. : Journal Press, etc*. 1955;40(2):351.
38. Lennard H, Jarvik ME, Abramson HA. Lysergic Acid Diethylamide (LSD-25): XII. A Preliminary Statement of its Effects Upon Interpersonal Communication. *The Journal of Psychology*. 1956;41(1):185.
39. Slater PE, Morimoto K, Hyde RW. The effects of LSD upon group interaction. *Arch Gen Psychiatry*. 1963;8:564.
40. Stoll A, Rothlin E, Rutschmann J, Schalch WR. Distribution and fate of ¹⁴C-labeled lysergic acid diethylamide (LSD 25) in the animal body. *Experientia*. 1955;11(10):396.
41. Axelrod J, Brady RO, Witkop B, Evarts EV. Metabolism of Lysergic Acid Diethylamide. *Nature*. 1956;178(4525):143.

42. Axelrod J, Brady RO, Witkop B, Evarts EV. The distribution and metabolism of lysergic acid diethylamide. *Ann N Y Acad Sci.* 1957;66(3):435.
43. Siddik ZH, R.D B, Graham Dring L, Smith RL, Tecwyn Williams R. The fate of lysergic acid di[14C]ethylamide ([14C]LSD) in the rat, guinea pig and rhesus monkey and of [14C]iso-LSD in rat. *Biochem Pharmacol.* 1979;28:3093.
44. Boyd ES. Metabolism of lysergic acid diethylamide. *Arch. Int. Pharmacodyn.* 1959;120:292.
45. Kiechel J-R. Pharmacocinétique et métabolisme des dérivés de l'ergot. *J.Pharmacol. (Paris).* 1979;10:533.
46. Poch GK, Klette KL, Hallare DA, Manglicmot MG, Czarny RJ, McWhorter LK, et al. Detection of metabolites of lysergic acid diethylamide (LSD) in human urine specimens: 2-oxo-3-hydroxy-LSD, a prevalent metabolite of LSD. *J Chromatogr B Biomed Sci Appl.* 1999;724(1):23.
47. Niwaguchi T, Inoue T, Nakahara Y. Studies on enzymatic dealkylation of D-lysergic acid diethylamide (LSD). *Biochem Pharmacol.* 1974;23(6):1073.
48. Aghajanian GK, Bing OH. Persistence of lysergic acid diethylamide in the plasma of human subjects. *Clin Pharmacol Ther.* 1964;5:611.
49. Upshall DG, Wailling DG. The determination of LSD in human plasma following oral administration. *Clin.chim.Acta.* 1972;36:67.
50. Levy G, Gibaldi M, Jusko WJ. Multicompartment pharmacokinetic models pharmacologic effects. *J.pharm.Sci.* 1969;50:422.
51. Wagner JG, Aghajanian GK, Bing OH. Correlation of performance test scores with "tissue concentration" of lysergic acid diethylamide in human subjects. *Clinical Pharmacology & Therapeutics.* 1968;9(5).
52. Levy G. Pharmacokinetics of LSD effect. *Clinical Pharmacology & Therapeutics.* 1969;10(1):134.
53. Tonini G, Montanari C. Effetti psichici della monoetilamide dell'acido lisergico (LAE32). *Giorn.psichiatri.Ferrara.* 1955;83:355.
54. Cai J, Henion J. Elucidation of LSD in vitro metabolism by liquid chromatography and capillary electrophoresis coupled with tandem mass spectrometry. *J Anal Toxicol.* 1996;20(1):27.
55. Canezin J, Cailleux A, Turcant A, Le Bouil A, Harry P, Allain P. Determination of LSD and its metabolites in human biological fluids by high-performance liquid

chromatography with electrospray tandem mass spectrometry. *J Chromatogr B Biomed Sci Appl.* 2001;765(1):15.

56. Schmid Y, Enzler F, Gasser P, Grouzmann E, Preller KH, Vollenweider FX, et al. Acute Effects of Lysergic Acid Diethylamide in Healthy Subjects. *Biol Psychiatry.* 2015;78(8):544.

57. Gasser P. Die Psycholytische Therapie in der Schweiz von 1988-1993. *Schweiz Arch Neurol Psychiatr.* 1996;147:59.

58. Sewell RA, Halpern JH, Pope HG, Jr. Response of cluster headache to psilocybin and LSD. *Neurology.* 2006;66(12):1920.

59. Karst M, Halpern JH, Bernateck M, Passie T. The non-hallucinogen 2-bromo-lysergic acid diethylamide as preventative treatment for cluster headache: an open, non-randomized case series. *Cephalalgia.* 2010;30(9):1140.

60. Krebs TS, Johansen PO. Lysergic acid diethylamide (LSD) for alcoholism: meta-analysis of randomized controlled trials. *J Psychopharmacol.* 2012;26(7):994.

61. Gasser P, Holstein D, Michel Y, Doblin R, Yazar-Klosinski B, Passie T, et al. Safety and efficacy of lysergic acid diethylamide-assisted psychotherapy for anxiety associated with life-threatening diseases. *J Nerv Ment Dis.* 2014;202(7):513.

62. EMCDDA. European Drug Report 2013. European Monitoring Center for Drugs and Drug Addiction (EMCDDA). 2013;www.emcdda.europa.eu.

63. Krebs TS, Johansen PO. Over 30 million psychedelic users in the United States. *F1000Res.* 2013;2:98.

64. Liakoni E, Dolder PC, Rentsch K, Liechti ME. Acute health problems due to recreational drug use in patients presenting to an urban emergency department in Switzerland. *Swiss Med Wkly.* 2015;145:w14166.

65. Dolder PC, Liechti ME, Rentsch KM. Development and validation of a rapid turboflow LC-MS/MS method for the quantification of LSD and 2-oxo-3-hydroxy LSD in serum and urine samples of emergency toxicological cases. *Anal Bioanal Chem.* 2015;407(6):1577.

66. Liakoni E, Dolder PC, Rentsch KM, Liechti ME. Presentations due to acute toxicity of psychoactive substances in an urban emergency department in Switzerland: a case series. *BMC Pharmacol Toxicol.* 2016;17(1):25.

67. Dolder PC, Liechti ME, Rentsch KM. Development and validation of an LC-MS/MS method to quantify LSD, iso-LSD, 2-oxo-3-hydroxy LSD, and nor-LSD and

identify novel metabolites in plasma samples in a controlled clinical trial.

Anal Bioanal Chem. 2017

68. Dolder PC, Schmid Y, Haschke M, Rentsch KM, Liechti ME. Pharmacokinetics and Concentration-Effect Relationship of Oral LSD in Humans. *Int J Neuropsychopharmacol*. 2015;19(1).
69. Liechti ME, Dolder PC, Schmid Y. Alterations of consciousness and mystical-type experiences after acute LSD in humans. *Psychopharmacology (Berl)*. 2016;10.1007/s00213-016-4453-0.
70. Dolder PC, Schmid Y, Muller F, Borgwardt S, Liechti ME. LSD Acutely Impairs Fear Recognition and Enhances Emotional Empathy and Sociality. *Neuropsychopharmacology*. 2016;41(11):2638.
71. Dolder PC, Schmid Y, Steuer AE, Kraemer T, Rentsch KM, Hammann F, et al. Pharmacokinetics and Pharmacodynamics of Lysergic Acid Diethylamide in Healthy Subjects. *Clin Pharmacokinet*. 2017;10.1007/s40262-017-0513-9.
72. Schmid Y, Hysek CM, Simmler LD, Crockett MJ, Quednow BB, Liechti ME. Differential effects of MDMA and methylphenidate on social cognition. *J Psychopharmacol*. 2014;28:847.
73. Hysek CM, Schmid Y, Simmler LD, Domes G, Heinrichs M, Eisenegger C, et al. MDMA enhances emotional empathy and prosocial behavior. *Soc Cogn Affect Neurosci*. 2014;9:1645.
74. Liechti ME, Schmid Y, Dolder PC, Hysek C. Effects of MDMA on social cognition. *European Neuropsychopharmacology: Elsevier*; 2016. p. S248.
75. Carhart-Harris RL, Kaelen M, Bolstridge M, Williams TM, Williams LT, Underwood R, et al. The paradoxical psychological effects of lysergic acid diethylamide (LSD). *Psychol Med*. 2016;46(7):1379.
76. Preller KH, Pokorny T, Krähenmann R, Dziobek I, Stämpfli P, Vollenweider FX. The Effect of 5-HT_{2A/1a} Agonist Treatment On Social Cognition, Empathy, and Social Decision-making. *European Psychiatry*; //2015. p. 22.
77. Komater M, Schmidt A, Bachmann R, Studerus E, Seifritz E, Vollenweider FX. Psilocybin biases facial recognition, goal-directed behavior, and mood state toward positive relative to negative emotions through different serotonergic subreceptors. *Biol Psychiatry*. 2012;72(11):898.

78. Mueller F, Lenz C, Dolder PC, Lang UE, Schmidt A, Liechti ME, et al. Acute effects of LSD on amygdala activity during processing of fearful stimuli in healthy subjects. *Transl Psychiatry*. 2017;doi:10.1038/tp.2017.54(7).
79. Steuer AE, Poetzsch M, Stock L, Eisenbeiss L, Schmid Y, Liechti ME, et al. Development and validation of an ultra-fast and sensitive microflow liquid chromatography-tandem mass spectrometry (MFLC-MS/MS) method for quantification of LSD and its metabolites in plasma and application to a controlled LSD administration study in humans. *Drug Test Anal*. 2016;10.1002/dta.2042.
80. Tagliazucchi E, Roseman L, Kaelen M, Orban C, Muthukumaraswamy SD, Murphy K, et al. Increased global functional connectivity correlates with LSD-induced ego dissolution. *Curr Biol*. 2016;26(8):1043.
81. Kaelen M, Roseman L, Kahan J, Santos-Ribeiro A, Orban C, Lorenz R, et al. LSD modulates music-induced imagery via changes in parahippocampal connectivity. *Eur Neuropsychopharmacol*. 2016;26:1099.
82. Carhart-Harris RL, Muthukumaraswamy S, Roseman L, Kaelen M, Droog W, Murphy K, et al. Neural correlates of the LSD experience revealed by multimodal neuroimaging. *Proc Natl Acad Sci U S A*. 2016;113:4853.
83. Terhune DB, Luke DP, Kaelen M, Bolstridge M, Feilding A, Nutt D, et al. A placebo-controlled investigation of synaesthesia-like experiences under LSD. *Neuropsychologia*. 2016;88:28.
84. Speth J, Speth C, Kaelen M, Schloerscheidt AM, Feilding A, Nutt DJ, et al. Decreased mental time travel to the past correlates with default-mode network disintegration under lysergic acid diethylamide. *J Psychopharmacol*. 2016;30(4):344.
85. Roseman L, Sereno MI, Leech R, Kaelen M, Orban C, McGonigle J, et al. LSD alters eyes-closed functional connectivity within the early visual cortex in a retinotopic fashion. *Hum Brain Mapp*. 2016;37(8):3031.
86. Lebedev AV, Kaelen M, Lovden M, Nilsson J, Feilding A, Nutt DJ, et al. LSD-induced entropic brain activity predicts subsequent personality change. *Hum Brain Mapp*. 2016;37(9):3203.
87. Kaelen M, Barrett FS, Roseman L, Lorenz R, Family N, Bolstridge M, et al. LSD enhances the emotional response to music. *Psychopharmacology (Berl)*. 2015;232(19):3607.

88. Griffiths RR, Johnson MW, Richards WA, Richards BD, McCann U, Jesse R. Psilocybin occasioned mystical-type experiences: immediate and persisting dose-related effects. *Psychopharmacology (Berl)*. 2011;218(4):649.
89. MacLean KA, Johnson MW, Griffiths RR. Mystical experiences occasioned by the hallucinogen psilocybin lead to increases in the personality domain of openness. *J Psychopharmacol*. 2011;25(11):1453.
90. Garcia-Romeu A, Griffiths RR, Johnson MW. Psilocybin-occasioned mystical experiences in the treatment of tobacco addiction. *Curr Drug Abuse Rev*. 2014;7(3):157.
91. Bedi G, Phan KL, Angstadt M, de Wit H. Effects of MDMA on sociability and neural response to social threat and social reward. *Psychopharmacology (Berl)*. 2009;207(1):73.
92. Kraehenmann R, Preller KH, Scheidegger M, Pokorny T, Bosch OG, Seifritz E, et al. Psilocybin-Induced Decrease in Amygdala Reactivity Correlates with Enhanced Positive Mood in Healthy Volunteers. *Biol Psychiatry*. 2015;78(8):572.

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Schmidt A , Mueller F, **Dolder PC**, Schmid Y, Zanchi D, Liechti M, Borgwardt S. Comparative effects of methylphenidate, modafinil and MDMA on response inhibition neural networks in healthy subjects. *Int J Neuropsychopharmacol*. 2017 May 19. doi: 10.1093/ijnp/pyx037.

Dolder PC, Liechti ME, Rentsch KM. Development and validation of an LC-MS/MS method to quantify LSD, iso-LSD, 2-oxo-3-hydroxy LSD, and nor-LSD and identify novel metabolites in plasma samples in a controlled clinical trial. *J Clin Lab Anal*. 2017 May 26. doi: 10.1002/jcla.22265

Binz TM, Williner E, Strajhar P, **Dolder PC**, Liechti ME, Baumgartner MR, et al. Chiral Analysis of Amphetamines in Hair by Liquid Chromatography-Tandem Mass Spectrometry: Compliance-Monitoring of attention deficit hyperactivity disorder (ADHD) patients under Elvanse(R) therapy and identification after controlled low dose application. *Drug Test Anal*. 2017;10.1002/dta.2208.

Mueller F, Lenz C, **Dolder PC**, Lang UE, Schmidt A, Liechti ME, et al. Acute effects of LSD on amygdala activity during processing of fearful stimuli in healthy subjects. *Transl Psychiatry*. 2017;doi:10.1038/tp.2017.54(7).

Kuypers PC, **Dolder PC**, Ramaekers JG, Liechti ME. Multifaceted empathy of healthy volunteers after single doses of MDMA: A pooled sample of placebo-controlled studies. *J Psychopharmacol*. 2017;https://doi.org/10.1177/026988111769.

Dolder PC, Holze F, Liakoni E, Harder S, Liechti ME. Alcohol acutely enhances decoding of positive emotions and emotional concern for positive stimuli and facilitates the viewing of sexual images. *Psychopharmacology (Berl)*. 2017;234(1):41.

Dolder PC, Schmid Y, Muller F, Borgwardt S, Liechti ME. LSD Acutely Impairs Fear Recognition and Enhances Emotional Empathy and Sociality. *Neuropsychopharmacology*. 2016;41(11):2638.

Dolder PC, Schmid Y, Steuer AE, Kraemer T, Rentsch KM, Hammann F, et al. Pharmacokinetics and Pharmacodynamics of Lysergic Acid Diethylamide in Healthy Subjects. *Clin Pharmacokinet*. 2017;10.1007/s40262-017-0513-9.

Stoller A, **Dolder PC**, Bodmer M, Hammann F, Rentsch KM, Exadaktylos AK, et al. Mistaking 2C-P for 2C-B: What a Difference a Letter Makes. *J Anal Toxicol*. 2017;41(1):77.

Liakoni E, **Dolder PC**, Rentsch KM, Liechti ME. Presentations due to acute toxicity of psychoactive substances in an urban emergency department in Switzerland: a case series. *BMC Pharmacol Toxicol*. 2016;17(1):25.

Liakoni E, **Dolder PC**, Rentsch K, Liechti ME. Acute health problems due to recreational drug use in patients presenting to an urban emergency department in Switzerland. *Swiss Med Wkly*. 2015;145:w14166.

Liechti ME, **Dolder PC**, Schmid Y. Alterations of consciousness and mystical-type experiences after acute LSD in humans. *Psychopharmacology (Berl)*. 2016;10.1007/s00213-016-4453-0.

Mauermann E, Filitz J, **Dolder P**, Rentsch KM, Bandschapp O, Ruppen W. Does Fentanyl Lead to Opioid-induced Hyperalgesia in Healthy Volunteers?: A Double-blind, Randomized, Crossover Trial. *Anesthesiology*. 2016;124(2):453.

Mueller F, Lenz C, Steiner M, **Dolder PC**, Walter M, Lang UE, et al. Neuroimaging in moderate MDMA use: A systematic review. *Neurosci Biobehav Rev*. 2016;62:21.

Strajhar P, Schmid Y, Liakoni E, **Dolder PC**, Rentsch KM, Kratschmar DV, et al. Acute Effects of Lysergic Acid Diethylamide on Circulating Steroid Levels in Healthy Subjects. *J Neuroendocrinol*. 2016;28(3):12374

Dolder PC, Schmid Y, Haschke M, Rentsch KM, Liechti ME. Pharmacokinetics and Concentration-Effect Relationship of Oral LSD in Humans. *Int J Neuropsychopharmacol*. 2015;19(1).

Dolder PC, Liechti ME, Rentsch KM. Development and validation of a rapid turboflow LC-MS/MS method for the quantification of LSD and 2-oxo-3-hydroxy LSD in serum and urine samples of emergency toxicological cases. *Anal Bioanal Chem*. 2015;407(6):1577.